INTERACTIVE EFFECTS OF HEMLOCK MORTALITY AND NITROGEN AVAILABILITY ON NUTRIENT POOLS AND FLUXES IN THE SOUTHERN APPALACHIAN MOUNTAINS

BY

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THESIS

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Abstract

The impacts of exotic insects and pathogens on forest ecosystems are increasingly recognized, yet the factors influencing the magnitude of effects remain poorly understood. Eastern hemlock (*Tsuga canadensis*) exerts strong control on nitrogen (N) dynamics, and its loss due to infestation by the hemlock woolly adelgid (*Adelges tsugae*) is expected to affect nutrient dynamics in impacted stands. I evaluated the potential for variation in N availability to influence the magnitude of effects of hemlock decline on N and P dynamics in mixed hardwood stands. I measured N and P pools and fluxes at three elevations (low, mid, high) subjected to increasing atmospheric N deposition where hemlock was declining or absent (as reference), in western North Carolina. Nutrient pools varied substantially with elevation and increasing N availability; total forest floor and mineral soil N increased ($p<0.0001$, $p=0.0017$, resp.) and forest floor and soil carbon (C) to N ratio decreased with elevation ($p<0.0001$, $p=0.0123$, resp.), suggesting that these high elevation pools are accumulating available N. Total extractable soil P was similar across the study area, however, P fractionation revealed distinct changes in the distribution of soil P fractions as N increased. Soils from high elevation, high N-available sites had 310% higher concentration of organic P and 55% smaller residual and refractory P pools than soils from low elevation, low N-available stands, suggesting that increased N availability has driven the depletion of recalcitrant P pools by stimulating biotic demand. Contrary to expectations, subsurface leaching of inorganic N was minimal overall (<1 kg ha^{-1} 9 mo^{-1}), and was not higher in stands with hemlock mortality. Across all elevation classes, hardwood foliar N:P ratios were lower in stands with declining hemlocks, suggesting trees are incorporating available P into biomass. Higher foliar N and P concentrations as well as observed increases in the growth of hardwood species in high elevation stands post-HWA infestation suggest that hemlock decline

has stimulated nutrient uptake by healthy vegetation within this mixed forest, and may thereby contribute to decoupling the relationship between N deposition and ecosystem nutrient loss.

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Abbreviations

DH	Hardwood stands intermixed with declining or dead eastern hemlock (*Tsuga canadensis* (L.) Carr.)
REF	Reference stands with similar hardwood species compositions that lack eastern hemlock
HWA	Hemlock woolly adelgid (*Adelges tsugae* Annand)
NO_3-N	Nitrogen as nitrate + nitrite (NO_3-N+ NO_2-N) as determined by a cadmium column reduction method on a Lachat QuikChem 8000 (Hach Co., Loveland, CO)
NH_4-N	Nitrogen as ammonium (NH_4-N, determined by the ammonia phenolate method on a Lachat QuikChem 8000 (Hach Co., Loveland, CO)
DRP	Dissolved reactive phosphorus as determined by an ortho-phosphate (PO_4-P) method on a Lachat QuikChem 8000 (Hach Co., Loveland, CO)
IER	Ion-exchange resin lysimeter as described by Susfalk and Johnson 2002
EGS	Early growing season, April 2010-July 2010
LGS	Late growing season, July 2010-October 2010
F/W	Fall/Winter, October 2010-February 2011
L or LOW	Low elevation class, 698-716 meters above sea level
M or MID	Middle elevation class, 917-943 meters above sea level
H or HIGH	High elevation class, 1402-1433 meters above sea level
BAI	Basal area index. Basal area (m^2) of all trees in a plot for all years 1991-2010

Introduction

Hemlock Woolly Adelgid

Exotic insects and pathogens pose a serious threat to forest ecosystems (Liebhold et al. 1995, Orwig and Foster 1998, Kizlinski et al. 2002, Ellison et al. 2005, Lovett et al. 2006, Loo 2009, Nuckolls et al. 2009, Gandhi and Herms 2010), and have been particularly destructive in the forests of eastern North America. One such example is the hemlock woolly adelgid (*Adelges tsugae*; HWA), an invasive species that has lead to the death of thousands of eastern hemlock (*Tsuga Canadensis*) and Carolina hemlock (*Tsuga caroliniana*) across the eastern United States (McClure 1990, Orwig and Foster 1998). Tree mortality can occur in as little as 4 years after infection, and across its native range, hemlock stands have rapidly succumbed to infestation and have not regenerated (Orwig and Foster 1998, Parker et al. 1998). Losing a late-successional species like the hemlock has major implications for forest ecosystems (Ellison et al. 2005, Preisser et al. 2008, Gandhi and Herms 2010, Nunez et al. 2010) such as changes in nutrient cycling, microclimate, light conditions (Jenkins et al. 1999, Lovett et al. 2002, Lovett et al. 2006), forest structure, productivity, nutrient uptake, and soil organic matter production and turnover (Lovett et al. 2006). In the southeast, hemlocks are most commonly found in riparian areas and therefore exert strong control over water and nutrient fluxes; their loss is expected to have major impacts on forest ecosystem function.

HWA was first documented in Virginia in 1951 (Parker et al. 1998, Evans and Gregoire 2007); the pest quickly spread northeast due to the density of its host tree (Morin et al. 2009), infesting and killing hemlocks of all sizes and preventing germination from existing seed banks (Orwig and Foster 1998). Since 1990, HWA spread has been approximately 12.5 km yr^{-1} in the northeastern US, and 15.6 km yr^{-1} in the southeast, suggesting that expansion rate is affected by

cold winter temperatures, low average annual daily minimum temperatures, high elevations, and long periods of low temperatures (Evans and Gregoire 2007). In 2003 HWA was spotted on trees as far south as western North Carolina and northern Georgia (Nuckolls et al. 2009). Here, the absence of cold winter temperatures enable HWA to overwinter and have led to rapid and extensive hemlock loss and predicted hemlock elimination (Trotter and Shields 2009).

Hemlock was previously lost from forest ecosystems during the Holocene, likely because of an insect invasion. The fossil pollen record in eastern North America showed a rapid decline of eastern hemlock in 4850 ^{14}C BP (Allison et al. 1986). The decline was synchronous throughout the range of hemlock (within the limits of ^{14}C dating) (Davis et al. 1980, Webb 1982, Allison et al. 1986), and occurred throughout a large region (New Hampshire to Michigan) where different climatic factors limited hemlock's growth and reproduction (Davis et al. 1980, Allison et al. 1986). The concentration of pollen of successional trees (birch, maple, beech, oak, eastern white pine) increased in sediment deposited immediately after the decline and was followed by increased pollen from trees that competed with hemlock in mature forests (Brugam 1978, Davis 1981, Davis 1983, Allison et al. 1986). Pre-decline levels of hemlock pollen were reached approximately 500 years after recovery began and 1900 years after the initial decline (Allison et al. 1986).

Currently, HWA co-occurs with populations of *Tsuga* (spp.) across the world, however, no other populations are affected to the point of widespread mortality as witnessed in the eastern U.S. (Havill et al. 2006). Havill et al. (2006) found the origin of the HWA in the eastern U.S. was from a single population native to southern Japan. Although a single haplotype was shared among all samples collected in eastern North America, it is presumed that western hemlock (*Tsuga heterophylla*) does not experience complete mortality from the population of HWA that

co-occurs with it because these populations co-evolved and developed defenses to protect itself from such dramatic consequences (Havill et al. 2006).

Several studies have examined HWA-induced hemlock decline and resulting changes in ecosystem processes in the northeastern U.S. (Orwig and Foster 1998, Jenkins et al. 1999, Kizlinski et al. 2002, Orwig et al. 2002, Yorks et al. 2003, Ellison et al. 2005, Stadler et al. 2005, Eschtruth et al. 2006, Lovett et al. 2006, Stadler et al. 2006, Orwig et al. 2008), but only a few have studied effects in the hemlock's native southern range (Ford and Vose 2007, Nuckolls et al. 2009). Besides remarkably different forest compositions (Ellison et al. 2005), a recent study by O'Brien et al. (2011) found significantly different ectomycorrhizal colonies in forest soils of hemlock vs. hemlock/hardwood stands. Ecosystem responses are predicted to be different in both of these regions.

It is unclear how the HWA infestation and the resulting hemlock mortality will affect forest structure of the eastern U.S., but several factors (including abiotic stressors such as nutrient availability) will likely interact. There is evidence that the southern extent of the native range of hemlock trees will be lost more quickly than those in the northern range due to lack of cold winter temperatures that reduce adelgid populations (Orwig et al. 2002), however lower densities of hemlock in the southeast (Morin et al. 2009) may also dampen ecosystem response. My objective was to evaluate the impact of hemlock decline and loss on ecosystem function and how this may interact with pre-existing differences abiotic indices such as nutrient availability. I focused on N and P dynamics in the southern Appalachian Mountains; these forests are amazingly beautiful, complex, and provide many ecosystem services for humans and wildlife alike. Hopefully I have added to the knowledge base regarding ecosystem disturbances and interactions at regional scales.

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Chapter 1

Interactive Effects of Hemlock Mortality and Soil Nitrogen Availability on Nitrogen Pools and Fluxes in the Southern Appalachian Mountains

Abstract

The impacts of exotic insects and pathogens on forest ecosystems are increasingly recognized, yet the factors influencing the magnitude of effects remain poorly understood. Eastern hemlock (*Tsuga canadensis*) exerts strong control on nitrogen (N) dynamics, and its loss due to infestation by the hemlock woolly adelgid (*Adelges tsugae*) is expected to decrease N retention in impacted stands. I evaluated the potential for variation in N availability to influence the magnitude of effects of hemlock decline on N dynamics in mixed hardwood stands. I measured N pools and fluxes at three elevations (low, mid, high) subjected to increasing atmospheric N deposition where hemlock was declining or absent (as reference), in western North Carolina. N pools and fluxes varied substantially with elevation and increasing N availability. Total forest floor and mineral soil N increased ($p<0.0001$, $p=0.0017$, resp.) and forest floor and soil carbon (C) to N ratio decreased with elevation ($p<0.0001$, $p=0.0123$, resp.), suggesting that these high elevation pools are accumulating available N. Contrary to expectations, subsurface leaching of inorganic N was minimal overall (<1 kg ha^{-1} 9 mo^{-1}), and was not higher in stands with hemlock mortality. Mean subsurface flux was 0.16 ± 0.04 (SE) (kg N ha^{-1} $3mo^{-1}$) in reference and 0.17 ± 0.05 (kg N ha^{-1} $3mo^{-1}$) in declining hemlock stands. Moreover, although N loss increased with N availability in reference stands, there was no relationship between N availability and loss in stands experiencing hemlock decline. Higher foliar N and observed increases in the growth of hardwood species in high elevation stands suggest that hemlock decline has stimulated N uptake by healthy vegetation within this mixed

forest, and may thereby contribute to decoupling the relationship between N deposition and ecosystem N loss.

Introduction

Globally, introductions and outbreaks of exotic insects and pathogens are a serious threat to forest ecosystems (Liebhold et al. 1995, Orwig and Foster 1998, Kizlinski et al. 2002, Ellison et al. 2005, Lovett et al. 2006, Loo 2009, Nuckolls et al. 2009, Gandhi and Herms 2010). Forests of eastern North America, in particular, have undergone substantial alteration during the past century. Exotic pathogens nearly eliminated the American chestnut (*Castanea dentata*) and American elm *(Ulmus americana)*, and several recent insect invaders are causing widespread decline of their host species, including the emerald ash borer (*Agrilus Planipennis*) and the hemlock woolly adelgid (*Adeiges tsugae*; HWA) (Liebhold et al. 1995, Lovett et al. 2006).

The functional impacts of exotic insects and pathogens are increasingly recognized, with a growing number of studies documenting short- and long-term changes in forest structure and function as a result of pest- or pathogen-induced tree defoliation, loss of vigor, or death (Jenkins et al. 1999, Kizlinski et al. 2002, Ellison et al. 2005, Eschtruth et al. 2006, Lovett et al. 2006, Ford and Vose 2007, Orwig et al. 2008, Nuckolls et al. 2009). Short-term effects often include defoliation, changes in microclimate and/or light conditions, increases in coarse woody debris, and/or altered biogeochemical cycling and resource availability (Jenkins et al. 1999, Lovett et al. 2002, Orwig et al. 2002, Eschtruth et al. 2006, Lovett et al. 2006, Ford and Vose 2007, Gandhi and Herms 2010). Long term consequences can include altered foliage and litter quality, changes in soil organic matter production and turnover, shifts in species composition, altered ecological

interactions and ecosystem productivity (Stadler et al. 2005, Lovett et al. 2006, Gandhi and Herms 2010).

The factors influencing the magnitude of such effects have received much less attention. In their framework for understanding potential impacts of exotic pests and pathogens, Lovett and colleagues (2006) proposed organizing predictions around the features of the host species, namely its uniqueness in meaningful ecosystem processes such as seed production or nutrient cycling, its importance or dominance within a forest stand, and its phytosociology (i.e., whether the host grows in pure or mixed stands, its position in successional dynamics of the forest). Whereas the impacts of losing unique species have recently been considered (Ellison et al. 2005), there has been little empirical work examining how impacts vary with species dominance or phytosociology. To date, most of the studies examining ecosystem consequences of exotic insect or pathogen outbreaks have focused on single species stands or mixed stands where the host species is dominant. However, many exotic invaders and their hosts have large ranges extending beyond a single region to forests that differ vastly in composition and phytosociology.

One such example is the Eastern hemlock (*Tsuga canadensis*), with a distribution extending from the southern Appalachians to southern Canada and west to the central Lake states. It is currently declining throughout much of its eastern range due to infestation by an aphid-like insect native to Asia (Ellison et al. 2005). Hemlock is dominant in the northeast and co-dominant or a minor constituent in mixed-hardwood forests elsewhere. It is considered a foundation species because it exerts strong control on community dynamics and ecosystem function by regulating food webs, productivity, and the flux of water, energy and nutrients (Ellison et al. 2005). Previous studies conducted in monotypic hemlock stands show that loss of these influential trees can decrease nitrogen retention and potentially redefine ecosystem

structure and function for decades or longer (Ellison et al. 2005). However, in the mixed hardwood forests of the southeastern US, loss of hemlock may not have the large impacts observed in monotypic stands of the Northeast. Knoepp et al. (2011) found no differences in N mineralization rates or soil solution N concentrations among hemlock plots infested with HWA or nearby hardwood stands. They concluded that, as hemlock is replaced with hardwoods, nutrient cycling rates and processes will not be drastically altered (Knoepp et al. 2011).

In addition to the factors proposed by Lovett et al. (2006), there can exist substantial diffferences in abiotic conditions that may influence ecosystem response to pest- or pathogen-induced species decline. Anthropogenic increases in biologically reactive N in the biosphere, for example, have resulted in measurable changes in soil and forest floor N pools and C:N ratios (Aber et al. 2003). Within regions these differences can be pronounced becuase N deposition varies with elevation and topopgraphy, as well as proximity to industrial areas (Weathers et al. 2000, Weathers et al. 2006). In a study examining impacts of eastern hemlock mortality in a rural and an urban setting, Templer and McCann (2010) found the two sites responded to atmospheric N inputs differently. The urban forest had higher inputs and exports of N, but N was lost primarily via nitrification/nitrate leaching, suggesting that vegetation and the microbial community transformed atmospheric N before it was leached from the soil profile. Nitrate lost from their rural site had an isotope signature similar to precipitation, indicating most N did not undergo biological cycling. Although less N was leached in the rural site overall, slow vegetative growth and weak biotic demand for N limited the capacity of biotic sinks to keep pace with N inputs (Templer and McCann 2010).

Collectively, these findings suggest that both biotic and abiotic factors have a role in mitigating the impacts of tree mortality resulting from exotic invasion; however, the interactive

effects of such factors remain largely uncharacterized. The loss of a species that strongly regulates nutrient retention may have a greater impact on nutrient dynamics in stands with high initial N availability versus more N-limited stands. In contrast, biotic demand for nutrients may reduce the impact of species loss on nutrient retention regardless of exogenous inputs. As exotic introductions accelerate (Levine and D'Antonio 2003), there is a critical need to understand controls on invasive species impacts to anticipate how forest ecosystems will change in the future.

The purpose of this study was to investigate potential abiotic and biotic controls on the biogeochemical impacts of an exotic insect. I evaluated N pools and fluxes in southern Appalachian forest stands subjected to increasing rates of N deposition where hemlock was absent (reference) or declining due to HWA to test the following hypotheses: (1) hemlock loss will decrease ecosystem N retention, with effects being less severe in the southern Appalachians compared to more northern forests because hemlock is sub-dominant and co-occurs with hardwoods not susceptible to attack by HWA, and (2) greater N availability will lower relative biotic demand for nitrogen, resulting in larger leaching losses of N in stands experiencing hemlock decline.

Methods

Site description and selection

This study was conducted at Coweeta Hydrologic Laboratory, an experimental forest in the southern Appalachian mountains of western North Carolina, USA. Prior to its acquisition by the Forest Service in 1934, areas within the Coweeta basin were burned semiannually by the Cherokee Indians, used for grazing or agriculture, and logged for *Castanea dentate* (DeVivo

1991, Elliott and Vose 2011). Today, the principal overstory species are of the genera *Quercus*, *Carya*, and *Liriodendron* with scattered groups of *Pinus rigida* and *T. canadensis* (Elliott and Vose 2011). Evergreen shrubs (*Rhododendron* and *Kalmia*) combine with *Cornus*, *Robinia*, *Acer*, and *Betula* to form a dense understory cover (Day et al. 1988, Elliott and Vose 2011). HWA was first documented in the Coweeta basin in 2003, with extensive infestation by 2005 (Nuckolls et al. 2009). Tree mortality can occur in as little as four years (McClure 1991); in 2010, nearly 50% of the eastern hemlocks found within Coweeta Hydrologic Laboratory were observed to be dead (Ford et al. 2011). All eastern hemlocks measured in this study were still standing, but visually estimated to be between 95-100% mortality. Annual precipitation is ~1900 mm with >100 mm occurring in most months. The growing season extends from early May to early October. Mean monthly temperatures are highest in June through August (~20^{o}C) and lowest in December through January (~5^{o}C) (Knoepp et al. 2008). Soils at Coweeta are mostly Inceptisols or Ultisols; parent material consists of high-grade metamorphic rocks (i.e., mica gneiss, mica schist) and metasedimentary rocks (i.e., metasandstone, phyllite, shale) (Chapter 2). See Table 1 for specific site details.

I established six 20 m x 20 m (0.04 ha) study plots at each of three elevations; low (698-716 m), mid (917-943 m), and high (1,402-1,433 m) experiencing increasing rates of atmospheric N deposition (and presumably N availability) (n=18 plots total). Plots at each elevation had a similar composition of mixed hardwoods, but half of them contained several (n= 5-31) dead or dying eastern hemlock trees (declining hemlock; DH) and the other half contained no hemlock trees and were used as reference (REF) (Table 1). Only stands lacking or with very small amounts of *Rhododendron maxima* were selected to avoid biasing nutrient cycling measurements (Boettcher and Kalisz 1990).

Vegetation measurements

All seedlings (<0.5 m) and saplings (>0.5 m) were counted within a 1 m x 20 m section on the western-most side of each plot. In the summer of 2010, trees and shrubs >1.37 m height and ≥2.5 cm dbh (diameter at 1.37 m height) were measured and identified to species with the exception of *Carya* spp. which were identified to genus.

Foliar samples from the mid-crown of the three dominant hardwood species in each plot (determined by total basal area) were taken using a sling-shot and/or pole pruner between 21 July 2010 and 4 August 2010. Three to five individuals per species were sampled; if fewer individuals of a species existed in the plot, less than three were sampled. Samples were composited by species, air dried, and ground to <1 mm using a Wiley mill, then a sub-sample was taken for chemical analysis. The mass of foliage (kg) was found using allometric equations (Day and Monk 1974, Elliott et al. 2002) for each individual species in a plot. For each species, foliar N concentration was multiplied by kg of foliage, and summed for a plot. This weighting technique allowed for comparison of foliar chemistry at the plot scale, despite differences in the species sampled and individual tree characteristics (e.g., age) within and across plots.

Forest floor (O horizons + decaying wood) was sampled in March 2010 for determination of total mass and pools of carbon (C) and N. I collected five samples per plot from a 0.09 m^2 quadrat of forest floor separated into Oi, Oe, and Oa horizons and wood (< 10 cm diameter). The samples were placed in paper bags, oven-dried to a constant weight at 60°C and weighed. Samples were composited by plot and layer, ground to <1 mm using a Wiley mill, and a sub-sample was taken for chemical analysis. Forest floor and foliar samples were analyzed for total C and N by combustion on a Flash EA 1112.

Basal area increment (BAI) cores were taken in October 2010 and read using a uni-slide Velmex measuring system (Velmex, Inc. Bloomfield, NY 14469, USA). I determined annual BAI from the difference of successive annual growth rings dating back to 1991 (14 years pre-infestation). BAI of all trees was expected to be highly dynamic among years, and inter-annual variability in tree growth is highly reflective of climatic conditions (Fritts 1972, Ford et al. 2011). To isolate differences due to infestation and N availability rather than climate, I followed the approach of Nuckolls et al. (2009) and Ford et al. (2011) and calculated ratios of those hardwood species occurring both in DH and REF plots (mean BAI in DH [cm^2] / mean BAI in REF [cm^2]) at each elevation.

Soil and environmental measurements

Twenty to twenty-five soil cores (2.0 cm diameter) at depths of 0-10 cm and 10-30 cm were taken from random locations within each plot in June 2010. Samples were stored in plastic bags and placed in coolers on ice during transport to the laboratory the same day. Soil samples were air-dried, sieved (2 mm), and homogenized prior to sub-sampling for analysis. Bulk density (g soil cm^{-3}) (both coarse fragment and < 2 mm fractions) was determined on soils from each plot using 4.3 cm diameter PVC cores to sample 0-10 cm and 10-30 cm depths. These data were used to calculate soil nutrient pools in kg ha^{-1}. Soil chemical analyses included total C and N combustion on a Flash EA 1112 and 0.01 M $CaCl_2$ soil pH (Deal et al. 1996).

Ion-exchange resin lysimeters (IERs) (Susfalk and Johnson 2002) were installed in April 2010 to collect inorganic N (NH_4-N + NO_3-N) from forest floor and subsoil leachate. These IERs were constructed using 2" PVC couplings with 25g of ion-exchange resin (Rexyn I-300 HOH Beads- Research Grade/Certified) resting between two layers of washed sand. Eight IERs were

randomly located throughout each plot: four below the forest floor ("forest floor lysimeters") and four below the major rooting zone ("mineral soil lysimeters"). To place the four mineral soil lysimeters, two 50-cm deep pits were dug with two channels perpendicular at the bottom of the pit. Two IERs were placed at the ends of these channels; far enough away from the loose soil to ensure the soil column above the lysimeters remained undisturbed. During quarterly sampling (July 2010, October 2010, February 2011), both forest floor and mineral soil lysimeters were swapped with freshly prepared IERs, stored in individual bags, and kept cool during transport to the laboratory. Initial placement of all IERs was randomized by maintaining a distance of ~1-2 m away from any tree trunk or shrub. After removal from the field, resin was extracted using 2M KCl (Langlois et al. 2003), and the extract was filtered through 0.7 um Whatman filter paper and frozen until analysis. Extracts were analyzed for NH_4-N using the phenolate method and NO_3-N + NO_2-N using a cadmium column reduction on a Lachat QuikChem 8000 (Hach Company, Loveland, CO). Total inorganic N flux was calculated by summing NH_4-N and NO_3-N, multiplying by the mL of KCl extractant used and the area of the lysimeter opening, and scaling up to kg ha^{-1}.

Total wet inorganic N deposition (NH_4-N + NO_3-N) was calculated using precipitation depth (cm) and chemistry data from Coweeta rain gauges SRG19 and 20 for low elevation sites, SRG 2, 40, and 45 for mid elevation sites, and SRG 31 for high elevation sites (Knoepp et al. 2008) (Table 1). Dry deposition and cloud water deposition were not estimated for these sites, but are likely to increase substantially with elevation in mountainous terrain (Lovett et al. 1997, Weathers et al. 2006, Knoepp et al. 2008).

Mineral soil moisture and temperature were measured 5 cm below the soil surface using an ECH_2O Water & Temp Sensor (Decagon Devices, Pullman, WA) at each plot during

quarterly sampling (2009: October; 2010: January, April, July, October; 2011: February) except at high elevations during the winter when the top 5 cm was frozen and the probe could not be inserted.

Statistical analysis

Samples with a single collection date (foliage, forest floor, composite soil samples) were analyzed with ANOVA using elevation and vegetation type (DH, REF) as main and interactive (elevation × vegetation type) effects. For inorganic N flux, I used plot means for each temporal period and determined changes over time with a repeated measures statement using an unstructured covariance structure (un) in a generalized linear mixed model with plot (elevation × vegetation type) as the subject. For soil moisture and temperature, an auto-regressive (ar(1)) and heterogeneous auto-regressive (arh(1)) (respectively) covariance structure was applied to account for temporal autocorrelation. For all models, I used the Slice statement to evaluate simple main effects of elevation and vegetation type in the elevation × vegetation type interaction. For BAI ratios, the slope of pre-infestation (1991-2004) and post-infestation (2005-2010) growth was calculated using linear regression, and these slopes were tested for differences between time periods and among and elevation classes using a generalized linear mixed model and Slice statements to test for simple effects. Data were logarithmically transformed as necessary to meet assumptions of normality and equal variance. All analyses were conducted in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Wet inorganic N (NO_3-N + NH_4-N) deposition varied significantly across the basin and increased with elevation ($F_{2,18}$=34.41, p<.0001) from April 2010 - December 2011 (Fig. 1). When conditions allowed for moisture and temperature readings at all plots (October 2009 & 2010, July 2010), soil moisture did not differ across study plots; however, soil temperature decreased with increasing elevation ($F_{2,54}$=15.56, p<.0001) and was lower within DH stands ($F_{1,53}$=12.77, p=0.0028).

Vegetation measurements

Understory vegetation density generally declined with elevation (Table 1). Seedling density (#seedlings m^{-2}) ranged from 9.39 ± 2.52 (mean ± standard error (s.e.)) in low elevation stands to 7.78 ± 1.14 in mid, to 5.82 ± 1.44 in high elevation stands. However, there was no significant difference in seedling density with elevation or between stand types, and no elevation × vegetation type interaction was apparent. Mid elevation stands exhibited a higher sapling density (0.72 ± 0.25 saplings / m^2) than low (0.50 ± 0.22) or high (0.37 ± 0.19) elevation stands ($F_{2,18}$=5.30, p=0.0224), but there was no difference between DH and REF plots, and no interaction.

Foliar N of hardwood species increased with elevation ($F_{2,18}$=17.52, p=0.0003) and was higher in DH stands ($F_{1,18}$=4.99, p=0.0454) (Fig. 2a). However, there was no interaction between elevation and vegetation type. The mass of total N in forest floor (Oi + Oe + Oa + wood) increased with elevation ($F_{2,18}$=49.36, p<.0001), consistent with increasing N availability (Fig. 2b). No differences were observed between DH and REF plots, and the interaction of elevation × vegetation type was not significant. Mean forest floor N in was 834 ± 48.6 kg ha^{-1} (s.e.) in high

elevation stands, 221 ± 37.4 kg ha^{-1} in mid elevations, and 205 ± 65.0 kg ha^{-1} in low elevations s.e. (Fig. 2b). Mean forest floor C was 19,659 ± 1,294.7 kg ha^{-1} (s.e.) in high elevation stands, 8,522.1 ± 1,045.4 kg ha^{-1} in mid elevations, and 10,329 ± 3,443.0 kg ha^{-1} in low elevations. This corresponded with a decrease in the total forest floor C:N molar ratio ($F_{2,18}$=52.54, p<.0001), which averaged 28 in high elevation plots, 46 at mid elevations, and 58 at low elevations However, there were no differences in forest floor C:N between vegetation types, and the elevation × vegetation type interaction was not significant.

Slopes calculated from BAI ratios showed that, prior to infestation, tree growth was similar across DH stands at different elevations (Fig. 3). Post-infestation, however, growth diverged among elevation classes ($F_{2,20}$=6.65, p=0.0093). Slopes were strongly positive in high elevation stands but close to zero or negative in mid and low elevation stands. Comparisons of slopes pre- versus post-infestation at each elevation indicated that tree growth increased at high elevations ($F_{1,18}$=4.27, p=0.0578), but remained similar at mid and low elevations following HWA infestation.

Soil and environmental measurements

Similar to forest floor N, total soil N increased with elevation in both the 0-10 cm ($F_{2,18}$=11.38, p=0.0017) and 10-30 cm ($F_{2,18}$=22.98, p<.0001) depths, but did not differ between DH and REF plots at either depth. The top 10 cm of soil contained 1,536 ± 106.1 (mean ± SE) kg N ha^{-1} and 25,493 ± 1,264 kg C ha^{-1} at low elevations and increased to 2,712 ± 213.4 kg N ha^{-1} and 43,319 kg C ha^{-1} at high elevations (data not shown). Mid elevations had 1,429 ± 173.7 kg N ha^{-1} and 27,262 ± 2,929 kg C ha^{-1}. Soil C:N molar ratio at the same depth was 19, 23, 20 at low,

mid, and high elevations, respectively ($F_{2,18}$=6.48, p=0.0123). The interactions term was not significant.

Solution entering the soil profile from just below the forest floor was dominated by NO_3-N during most of the sampling period, but was nearly equally comprised of NH_4-N and NO_3-N during the April-July period (Fig. 4). Inorganic N flux in surface lysimeters also increased with elevation but effects varied with time (elevation × temporal period: $F_{4,54}$=11.19, p=0.0004), such that flux increased with elevation in the early growing season ($F_{2,54}$=11.74, p=0.0015) and the fall/winter period ($F_{2,54}$=10.67, p=0.0022), but was equivalent across elevations during the late growing season (Fig. 4). The effect of elevation on inorganic N flux depended on stand type ($F_{2,54}$=3.29, p=0.0725). The simple main effect of elevation on inorganic N flux was appreciably stronger in REF stands ($F_{2,27}$=24.07, p<.0001) than in DH stands ($F_{2,27}$=5.65, p=0.0187), suggesting that increasing N availability was driving greater inorganic N flux in the forest floor of reference stands where hemlock was absent. Temporal period also modulated the effects of elevation on forest floor inorganic N flux in each vegetation type (elevation × veg. type × temporal period: $F_{4,54}$=2,99, p=0.0612). In DH stands, surface inorganic N flux increased with elevation during the fall/winter period ($F_{2,27}$=8.17, p=0.0058), but was similar across elevations during other periods. In REF stands, inorganic N flux increased with elevation during the early growing season ($F_{2,27}$=11.50, p=0.0016), but not during other periods.

Most N leached in soil solution was in the form of nitrate-N, but this amounted to less than 0.9 kg ha^{-1} at any one elevation over the entire 9-mo sampling period. Inorganic N in soil leachate also varied by temporal period ($F_{2,54}$=53.62, p<.0001) with relatively small amounts of inorganic N (<1 kg ha^{-1}, mostly NO_3-N) (Fig. 4) at any elevation during the sampling period. The relationship between inorganic N loss, elevation and time varied with vegetation type (Fig. 4),

such that elevation had a positive effect on inorganic N flux in REF stands during the late growing season ($F_{2,54}$=5.53, p=0.0199), and varying effects in DH stands at other times of the year (Fig. 5).

Discussion

The objective of this study was to evaluate the interactive effects of pest-induced tree decline and soil N availability on ecosystem N dynamics. I expected that increasing N availability would increase N loss as soil and biotic sinks became N saturated, and that hemlock mortality would enhance this effect. Although it appears that soils and vegetation have responded to elevation-driven increases in N availability by assimilating more N, the observed pattern of low subsurface leaching at all sites suggests that these sinks have not yet reached capacity. Moreover, there was also no evidence that tree mortality has enhanced N loss in this system. Hardwoods in declining stands at high elevations where N inputs were highest had two times higher foliar N concentration than reference stands at the same elevation, and increased their rate of growth following HWA infestation. Collectively, these results suggest that the strength of the biotic N sink has increased in stands with declining hemlock to the extent that available N is being taken up and assimilated into biomass rather than leached from the system.

Previous studies demonstrate that nitrification rates increase in stands infested with HWA regardless of hemlock percent mortality within two to three years of infestation (Jenkins et al. 1999, Orwig et al. 2008). Nitrogen leaching is therefore expected to increase following hemlock decline (Jenkins et al. 1999, Kizlinski et al. 2002). Indeed, Yorks et al. (2003) observed elevated N leaching within two to three months of girdling hemlocks to simulate HWA-induced mortality, and found that concentrations remained high relative to control stands for the duration of the

study. Working in the southern Appalachians, however, Knoepp et al. (2011) found minor effects of HWA infestation on N mineralization rates and soil solution N concentrations in plots where hemlock represented less than 50% of basal area. The greater relative importance of hemlock in New England forests may explain the general lack of agreement between the findings of previous studies and the results described here and in Knoepp et al. (2011).

In their framework for understanding the impacts of pest and pathogen outbreaks on forest ecosystems, Lovett et al. (2006) propose that the importance of the host species should influence how its loss impacts forest ecosystems. If a species is dominant in terms of its basal area or litter production, or unique with respect to its effects on ecosystem processes, its loss may cause more damage and alter conditions more prominently than the death of another species that is sub-dominant or exerts less control on ecosystems processes. In the southeastern US, hemlock is dominant in riparian areas and occurs as a co- or sub-dominant species where moisture and temperature conditions permit, but overall it is much less abundant than in New England and northern forests (Ellison et al. 2005, Morin et al. 2009). Despite being a sub-dominant species, hemlock has an important physiological role in southern Appalachian forests. Hemlock produce low quality litter (Lovett et al. 2004) which decomposes slowly (Mcclaugherty et al. 1985) and is associated with slow rates of nitrogen cycling (Finzi et al. 1998, Jenkins et al. 1999, Templer et al. 2003, Lovett et al. 2004, Templer and McCann 2010); although each index of the N cycle can respond differently, hemlock has thus been described as one of the most conservative with respect to nutrient cycling (Lovett et al. 2004). This characteristic may partly explain the higher N leaching rates I observed in low elevation stands compared with mid and high elevation stands during the fall/winter sampling period. My study plots represented the natural distribution of hemlock in the southeastern U.S.; hemlock comprised 23-56% of the relative basal area within

low elevation DH plots, 13-52% in mid elevation DH plots, and 5-8% in high elevation DH plots. These findings provide empirical support for the hypothesis that the impacts of a species loss on N dynamics will depend on its relative importance within a forest.

Nitrogen availability also influenced stand-level response to hemlock decline but not as predicted. The interaction plots (Fig. 5) demonstrate that N leaching is two to four times higher in high elevation reference stands than low elevation reference stands across all temporal periods. In contrast, the change in N leaching between low and high elevation declining hemlock stands is comparatively smaller and its direction varies with temporal period: positive during early growing season, neutral during late growing season, and negative during the fall/winter period. Nitrogen leaching in reference stands strongly mirrors the precipitation-driven N deposition pattern, in which N inputs are highest during early summer when precipitation is greatest and lowest during fall and winter when there is less precipitation. In high elevation declining hemlock stands, however, there is a decoupling of the relationship between N deposition and ecosystem N loss.

The observed trends are broadly consistent with the phenology of biological activity. For example, during the April-July period, similarly high rates of N loss in high elevation declining and reference stands may be attributable to the late onset of biological activity combined with high N deposition rates. During the July-October period, biological activity in high elevation stands should promote N uptake, resulting in relatively less N loss in declining than reference stands. Similarly low levels of leaching in high elevation reference and declining stands during the October-February period may be due to low deposition rates and low biotic demand of surrounding vegetation, whereas higher-than-expected levels at low elevations during this period may be due to large canopy gaps left by declining hemlock, which may allow sunlight to reach

the forest floor, thus encouraging faster snowmelt and greater N flushing. Experiments will be needed to test the generality of these conclusions and reveal the mechanisms underlying these patterns. Yet, my results are comparable to those of Templer and McCann (2010) who found that, following HWA infestation, biota can take up and transform much of the N found in soil solution even when N inputs are high.

The reasons that biotic demand was strong enough to decrease N leaching at high elevations but not at low elevations remain unclear. Nitrogen retention may be a function of the forest floor or soil C pool (Evans et al. 2006, Lovett and Goodale 2011), but also may be co-limited by phosphorus (P). Hemlock mortality may have enhanced P availability in high, but not low elevation stands. As discussed in Chapter 2, I found higher flux of dissolved reactive P in stands experiencing hemlock mortality, as well as increased foliar P concentration within high elevation (and high N-deposition) sites experiencing hemlock decline. If hemlock mortality increases P availability, there may be an increased demand for N due to stimulation of primary production. This agrees with the increase in BAI of hardwoods in high elevation declining stands.

Previous studies have suggested increases in biotic N pools may indicate symptoms of N excess in ecosystems (Aber et al. 1998, Fenn et al. 1998). However, Lovett and Goodale (2011) recently suggested that N can flow to all sinks and losses within a system simultaneously, with the fate and temporal patterns of flow of N dependent on the strength of the sinks and the factors that control them. For example, exogenous N may be transferred to biotic pools such as foliage or stored in wood which may increase forest floor N or it may be allocated for increased tree growth, even as some losses occur. Interpreted in this light, my results suggest that, although high elevation stands may be progressing toward N saturation, soil and vegetation sinks still have

sufficient capacity to store exogenous N. However, the observed differences in N leaching with temporal period demonstrate that sink strength can vary with other factors.

Previous work has also showed that biotic sinks can modulate the effects of high N availability. In an N and sulfur (S) addition study at Harvard Forest in Massachusetts,Magill et al. (1997) found that foliar and fine root N concentrations were elevated in N-treated plots, although N leaching generally increased with N inputs. Ecosystem N retention ranged from 93-97% (70-92% of inputs were retained in the soil pool) (Magill et al. 1997). Similarly, Boggs et al. (2005) found positive relationships between N deposition and basal area increase, foliar N concentrations, and forest floor N in southern Appalachian forests. Within this same region, Knoepp and Swank (1998) found that vegetation exerts more control on N mineralization and nitrification rates than does elevation.

Determining the effects of tree species loss may be more difficult in mixed stands due to their complex phytosociology. Loss of hemlock in the southeastern US will likely liberate less N compared to pure hemlock stands, despite the ability of hemlock to regulate N cycling. Moreover, changes in N dynamics will likely be gradual due to the relatively slow defoliation of dying hemlocks, and liberated N may be redistributed within the system rather than leached (Lovett et al. 2002). Differences in observed responses to hemlock mortality indicate the impacts of HWA will be manifested in different ways across the various ecosystems found within the native range of this species. The controls on these impacts will also likely depend on the strength of biotic sinks and how the nutrient cycling processes of these mixed deciduous forests interact with abiotic variables such as N availability. Magnitudes of impacts may depend on the species composition of affected stands. Given that exotic insects and pathogens are now a widespread, persistent problem in many forests, understanding the controls on impacts is important challenge.

Conclusion

Whereas eastern hemlock is functionally unique in regards to its N cycling, it is a sub-dominant species in southern Appalachians forests. My results suggest a paradox that hemlock decline has strengthened sinks under conditions of high N availability by increasing biotic N demand in hardwoods that co-occur in these mixed stands, even though hemlock loss has resulted in minimal changes in N dynamics elsewhere in this region. Overall, my findings suggest that the impacts of pest-induced tree decline on N retention will depend on the capacity of remaining biotic sinks to capture and sequester available N.

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Table 1 Site characteristics for the declining hemlock (DH) and reference (REF) plots at low (L), middle (M), and high (H) elevations, including dominant vegetation sampled for foliar analysis, elevation (m), atmospheric wet inorganic nitrogen deposition (kg NH_4-N + NO_3-N ha^{-1}) measured April 2010-December 2010, *Tsuga canadensis* % basal area per plot, and seedling/sapling densities.

Site	Dominant hardwoods sampled for analysis of foliar chemistry		Elevation (m)	Hemlock %BA per plot	Vegetation density (seedlings m^{-2})	(saplings m^{-2})
DH-L	*Acer rubrum* *Betula lenta* *Ilex opaca* *Liriodendron tulipifera*	*Nyssa sylvatica* *Oxydendrum arboreum* *Quercus alba*	710-716	23-56	12.68 ± 3.42	0.50 ± 0.22
REF-L	*Acer rubrum* *Betula lenta* *Carpinus caroliniana* *Fagus grandifolia*	*Liriodendron tulipifera* *Quercus alba* *Quercus velutina*	698-715	0	6.10 ± 3.02	1.13 ± 0.58
DH-M	*Acer rubrum* *Betula lenta* *Carya spp.*	*Liriodendron tulipifera* *Nyssa sylvatica* *Oxydendrum arboreum*	917-934	13-52	8.07 ± 1.74	0.72 ± 0.25
REF-M	*Acer rubrum* *Carya spp.* *Liriodendron tulipifera* *Oxydendrum arboreum*	*Quercus coccinea* *Quercus velutina* *Robinia pseudoacacia*	924-943	0-4	7.50 ± 1.83	2.72 ± 0.41
DH-H	*Acer pensylvanicum* *Acer rubrum* *Betula alleghaniensis*	*Fagus grandifolia* *Quercus rubrum*	1402-1433	5-8	6.83 ± 2.80	0.37 ± 0.19
REF-H	*Acer pensylvanicum* *Acer rubrum* *Acer saccharum*	*Betula alleghaniensis* *Ostrya virginiana* *Prunus serotina*	1411-1423	0	4.80 ± 1.21	0.22 ± 0.04

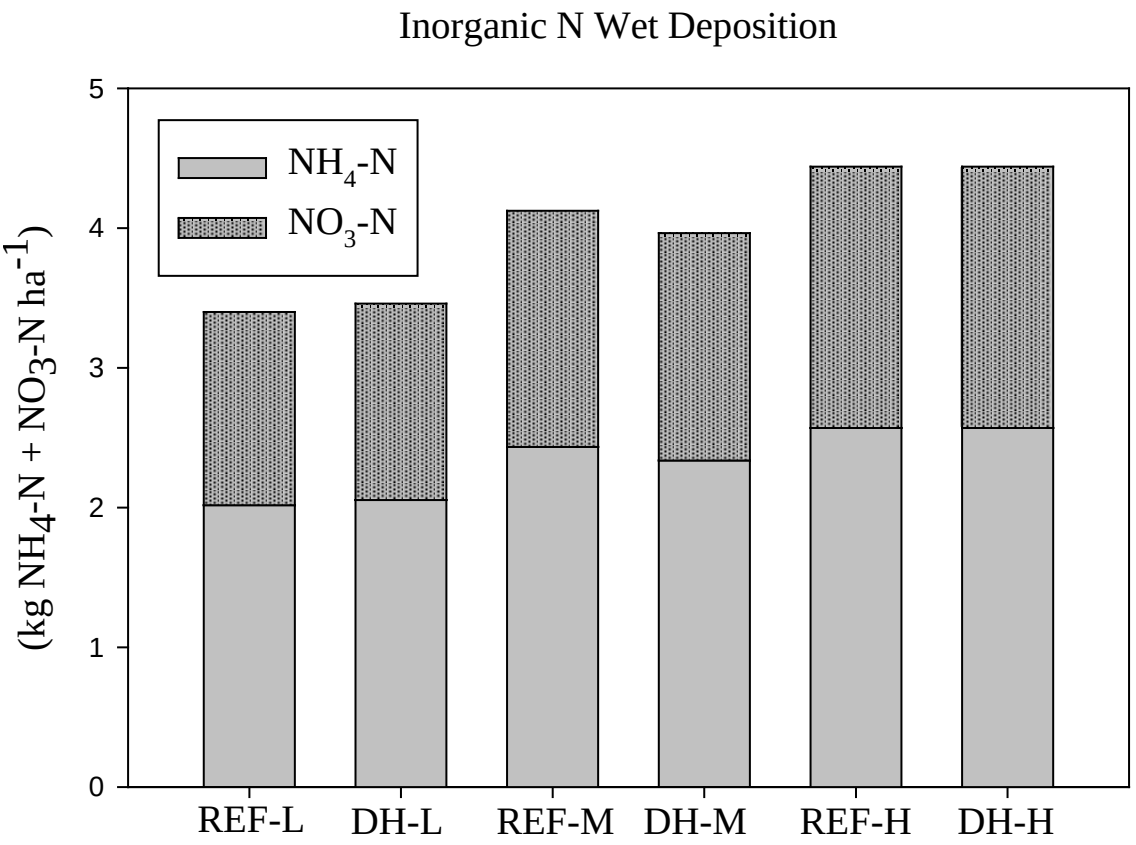

Figure 1 Total inorganic nitrogen wet deposition over nine months (April-December 2010) as measured by various rain gauges placed throughout the Coweeta basin in western North Carolina.

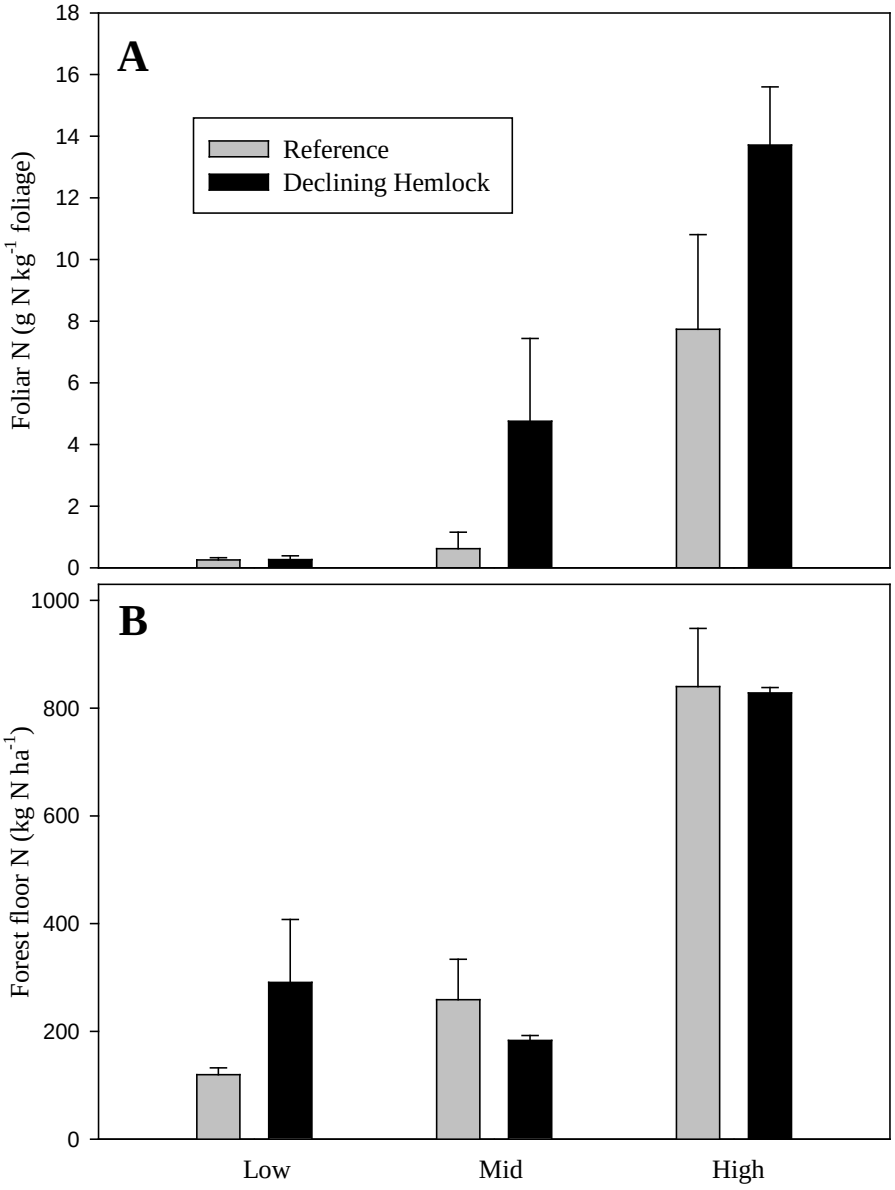

Figure 2 Foliar nitrogen (A) and total O horizon (Oi + Oe + Oa + wood) N (kg ha^{-1}) (B).

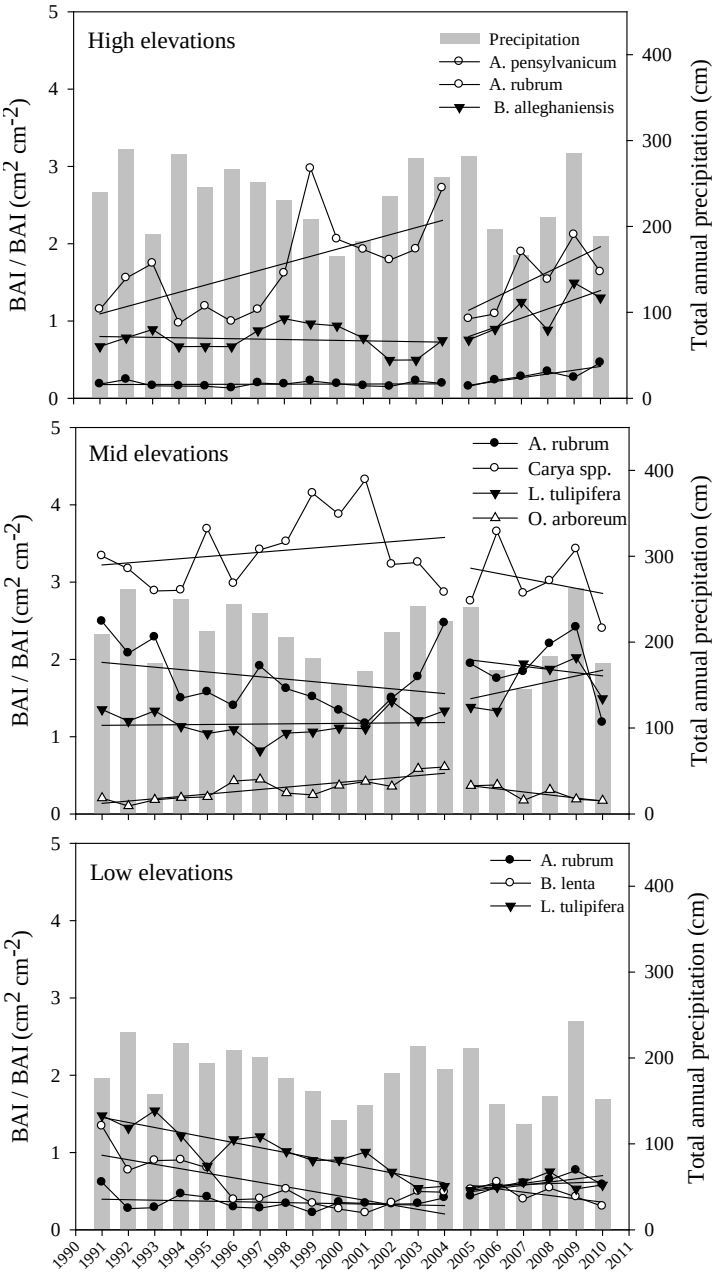

Figure 3 Mean annual basal area increment (BAI) of co-occurring hardwood trees in declining hemlock stands relative to the BAI of hardwood trees in reference stands for species occurring in both types of plots. Shaded bars represent total annual precipitation at rain gauges that most closely correspond to plot locations.

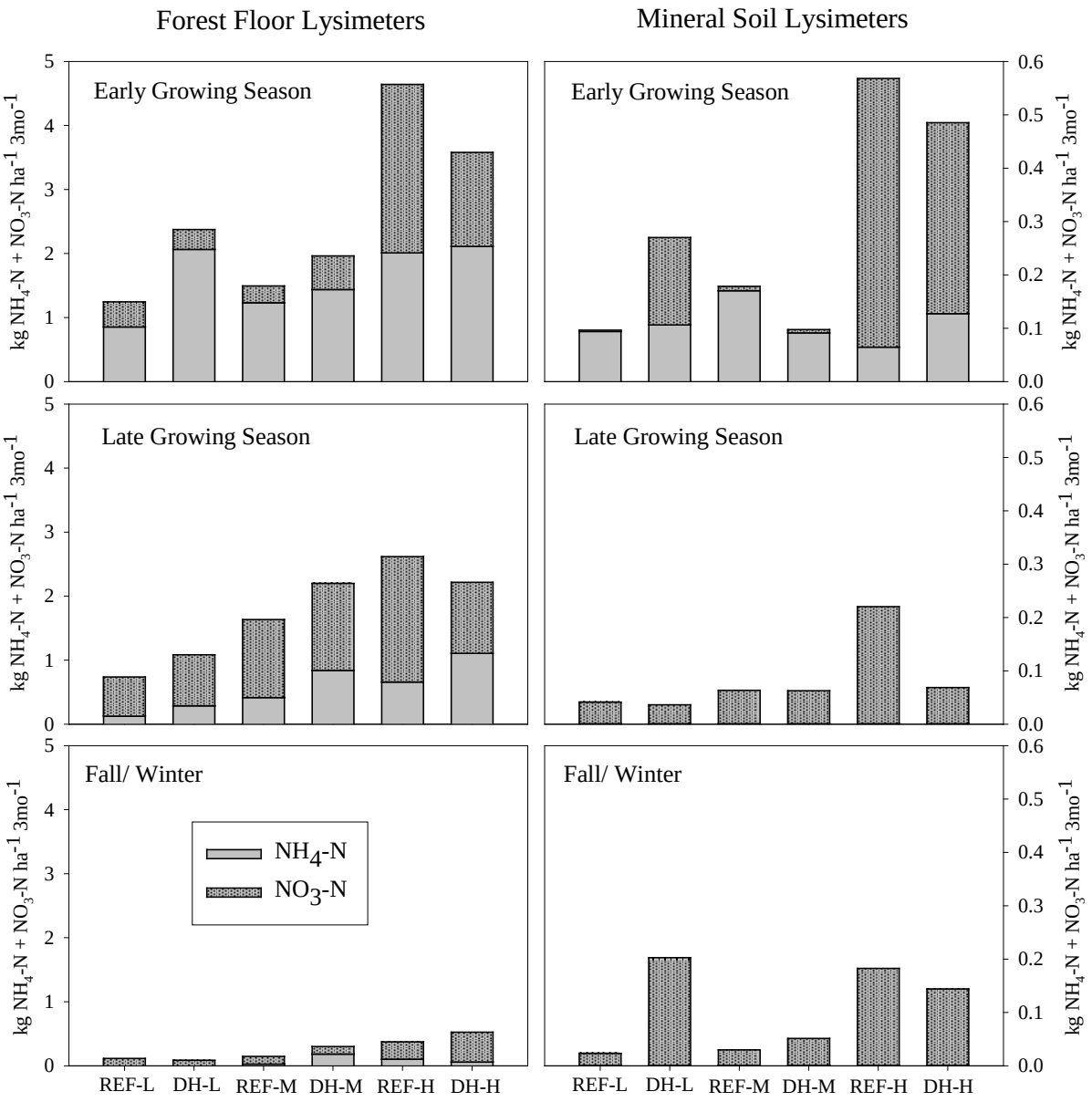

Figure 4 Inorganic N (kg NO_3-N+NH_4-N ha^{-1} $3mo^{-1}$) flux in declining hemlock (DH) and reference (REF) stands for the entire sampling period (April 2010- February 2011). Lysimeters were in-situ for three months (~100-day periods), represented by Early Growing Season (April-July 2010), Late Growing Season (July-October, 2010), and Fall/Winter (October 2010-February 2011).

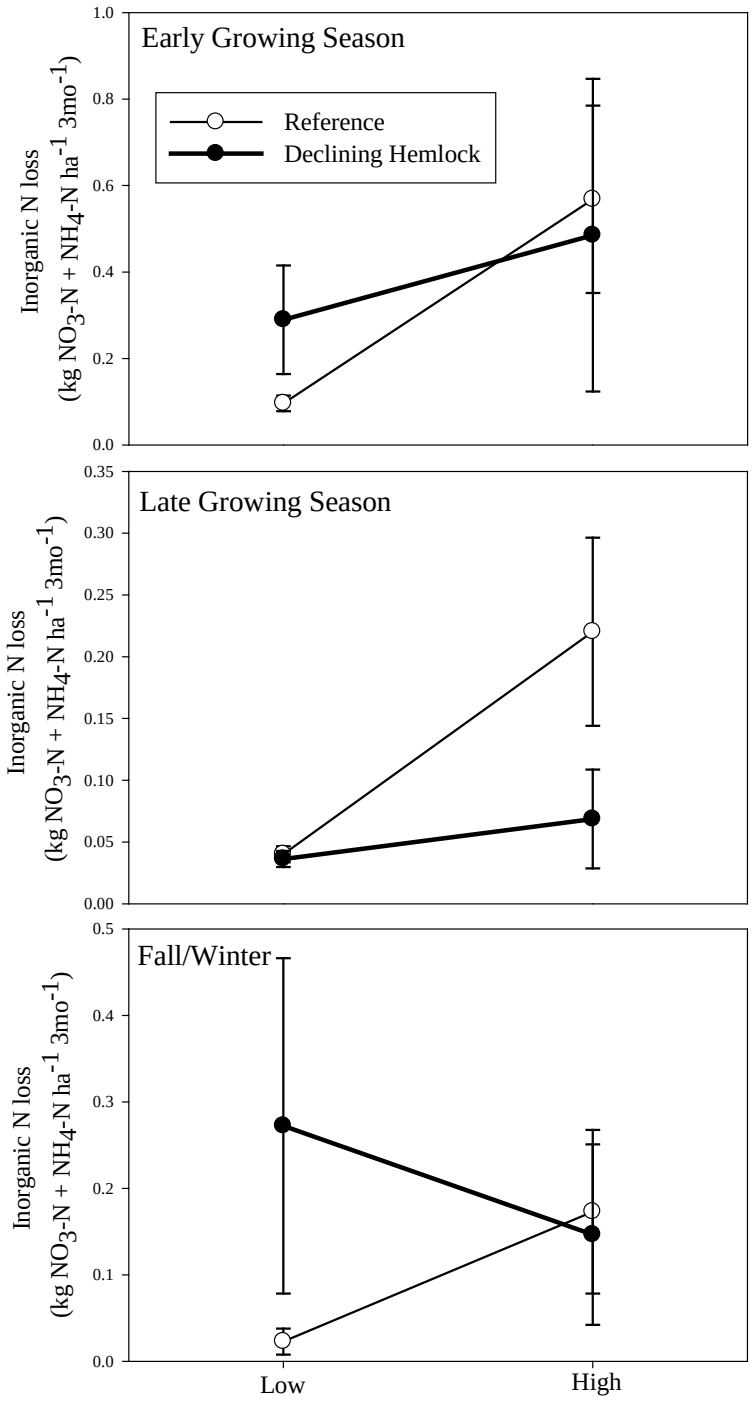

Figure 5 Interaction plots of inorganic N loss from low and high elevation stands during each sampling period. The y axes are different in order to best represent the patterns of leaching across elevations and between time periods.

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Chapter 2

Interactive Effects of Disturbance and Nitrogen Availability on Phosphorus Availability in the Southern Appalachian Mountains

Abstract

Understanding the short-term ecological controls on phosphorus (P) availability is increasingly important in light of the rapid pace at which human activities are altering biogeochemical cycles and potentially introducing P limitation. I measured P pools and fluxes in eighteen mixed forest stands at three elevations (low, mid, high) with increasing soil N availability, where hemlock (*Tsuga canadensis*) was absent or declining due to infestation by the exotic hemlock woolly adelgid (*Adelges tsugae*). While total soil P was similar across the study area, phosphorus fractionation revealed distinct changes in the distribution of soil P fractions as N increased. Soils from high elevation stands where N availability was greatest had 310% higher concentration of organic P and 55% smaller residual and refractory P pools than soils from low elevation stands where N availability was lower, suggesting that increased N availability has driven the depletion of recalcitrant P pools by stimulating biotic demand. These differences in P distribution influenced how tree mortality affected P dynamics. At high elevations, stands containing declining hemlocks had higher fluxes of P from the forest floor than reference stands at similar elevations ($p<0.05$), whereas, at low and mid-elevations, there were no consistent differences between stands. Across all elevation classes, hardwood foliar N:P ratios were lower in stands with declining hemlocks, suggesting trees are incorporating available P into biomass. Collectively, these results suggest that increased N availability enhances bioavailable P, which is sequestered in vegetation until disturbances liberate it.

Introduction

Phosphorus (P) is critical to biotic function and essential to the development and maintenance of ecosystems (Walker and Syers 1976, Crews et al. 1995, Wardle et al. 2004, Richter et al. 2006, Buendia et al. 2010, Vitousek et al. 2010, Porder and Hilley 2011, Turner and Engelbrecht 2011). Decades of research show that geochemical processes regulate P availability over long time scales, with soil age, parent material mineralogy, and climate explaining most of the variation in the long-term distribution of soil P (Walker and Syers 1976, Treseder and Vitousek 2001, Buendia et al. 2010, Vitousek et al. 2010, Porder and Hilley 2011). In contrast, ecological processes mainly influence P availability over the short term, potentially resulting in proximate nutrient limitation over decades to millennia (Cross and Schlesinger 1995, Wardle et al. 2004, Vitousek et al. 2010). Because of the transitory and more localized nature of these effects on P availability (Austin and Vitousek 1998, Wardle et al. 2004, Richter et al. 2006, Vitousek et al. 2010) ecological processes have generally received less attention compared to geochemical processes.

Human activities are substantially altering global ecological processes which in turn may influence P availability. Consequently, such pathways may exert stronger and more persistent control on P pools and fluxes than previously appreciated. Human-enhanced nitrogen (N) deposition, for example, has chronically increased the supply of N to terrestrial and aquatic ecosystems (Vitousek et al. 1997), and recent evidence suggests these added inputs could induce P limitation (Elser et al. 2007, Gress et al. 2007). Two mechanisms could account for anthropogenically driven P limitation: (1) increased N availability could stimulate primary productivity, thereby increasing biotic P demand; or (2) low soil pH driven by acid-related deposition could cause mobilization of soil aluminum (Al) and iron (Fe) bound P, resulting in

increased P sorption and decreased P availability (Sherman et al. 2006). An important assumption underlying the first mechanism is that recalcitrant P fractions are not bioavailable. However, recent studies have shown added N does not induce P limitation (Finzi 2009, Weand et al. 2010) and even suggest that over time strong biotic sinks can drive the transfer of P from recalcitrant to labile pools. Comparing P fractions of archived soils from aggrading forests, Richter et al. (2006) found that biotic P demand drove the depletion of slowly cycling inorganic P pools, thereby maintaining the labile soil P fraction and overall P availability. Fertilization studies also support this finding, and show that increased N availability stimulates microbial and root production of the extracellular enzyme phosphatase, which cleaves ester-P bonds in soil organic matter and increases local P availability (McGill and Cole 1981, Olander and Vitousek 2000, Treseder and Vitousek 2001, Wang et al. 2007, Finzi 2009, Vitousek et al. 2010, Weand et al. 2010).

Human alteration of natural disturbance regimes also has the potential to produce changes in P turnover and availability. Most, if not all, ecosystems experience disturbance (White 1979); however, land conversion and introductions of non-native species are occurring at unprecedented rates and modifying disturbance frequency, extent and intensity (Levine and D'Antonio 2003, Turner 2010). Disturbances can positively or negatively affect P availability. Those that interrupt the nutritional demands of vegetation without removing biomass or soil (e.g., windthrow, pest or pathogen outbreaks) can initially rejuvenate available P pools, although the rapid successional biomass production that often occurs after large disturbance events may sequester available P (Wardle et al. 2004, Swanson et al. 2011). On the other hand, landscapes experiencing disturbances that remove large amounts of fertile soil and/or biomass (e.g., landslides, agricultural land conversion) can experience P limitation (Matson et al. 1997, Frizano et al. 2002).

Anthropogenic N enrichment and disturbances can also operate together, yet few studies have considered how they interact to affect P availability. If greater N supply increases the pool of bioavailable P, the impact of disturbance on ecosystem P might be much greater in areas subjected to high rates of atmospheric N deposition. I hypothesized that in response to disturbance, forest stands with greater N availability, and potentially larger bioavailable P pools would experience larger perturbations to P pools and fluxes than areas with lower N availability. Moreover, because many ecosystems appear to be co-limited by N and P (Elser et al. 2007), vegetation response to disturbance might vary substantially and further alter nutrient cycling.

The objective of this study is to evaluate how interactions between N availability and forest disturbance, specifically the decline of eastern hemlock (*Tsuga canadensis*) due to exotic invasion by the hemlock wooly adelgid (HWA; *Adelges tsugae*), influence P availability in the southern Appalachian Mountains. Nitrogen deposition varies over relatively short distances within the southern Appalachians due to differences in precipitation caused by orographic lift. Previous work demonstrates that these differences produce substantial variation in N availability across the southern Appalachian landscape (Knoepp et al. 2008). Although many studies have investigated the effects of tree mortality on C and N cycles (Jenkins et al. 1999, Nuckolls et al. 2009, Knoepp et al. 2011), changes in P pools and fluxes remain poorly understood. Invasive pests and pathogen outbreaks are a constant and reoccurring issue in North America (Lovett et al. 2006), and ecosystem response to invasions and the consequences regarding P, an ultimate limiting nutrient (Vitousek et al. 2010), are critical to the understanding of forest ecosystem dynamics.

Methods

Site description and plot selection

This study was conducted at the Coweeta Hydrologic Laboratory, an experimental forest in the southern Appalachian mountains of western North Carolina, USA. HWA was first documented in the Coweeta basin in 2003, with extensive infestation and onset of hemlock decline by 2005 (Nuckolls et al. 2009). Annual precipitation is ~1900 mm with >100 mm occurring in most months, and varies with elevation. The growing season extends from early May to early October. Mean monthly temperatures are highest in June through August (~20^{o}C) and lowest in December through January (~5^{o}C) (Knoepp et al. 2008). Soils at Coweeta are mostly Inceptisols or Ultisols; parent material consists of high-grade metamorphic rocks (i.e., mica gneiss, mica schist) and metasedimentary rocks (i.e., metasandstone, phyllite, shale). See Table 2 for specific site details.

I established six 20 m x 20 m (0.04 ha) plots at each of three elevation classes (low, mid, high) (Table 2). Increasing elevation was associated with increasing levels of N deposition, such that deposition ranged from 3.40 ± 0.06 (mean ± SE) kg ha^{-1} in low elevation plots to 4.44 kg ha^{-1} at high elevation plots (Chapter 1). Within each elevation class, three of six plots contained dead or dying eastern hemlock trees (declining hemlock; DH), while the remaining three plots had a similar composition of mixed hardwoods and were used as reference (REF) (n=18 plots total). Only areas lacking or with very small amounts of *Rhododendron maxima* were selected to avoid biasing nutrient cycling measurements (Boettcher and Kalisz 1990).

Vegetation measurements

Trees >1.37 m height and ≥ 2.5 cm dbh (diameter at 1.37 m height) were identified to species with the exception of *Carya* spp., which were identified to genus. Foliar samples from the

mid-crown of the three dominant hardwood species (determined by total basal area) were taken using a sling-shot and/or pole pruner between 21 July 2010 and 4 August 2010. Three to five individuals per species were sampled; if fewer individuals of a species existed in the plot, less than three were sampled. Samples were composited by species, air dried, and ground to <1 mm using a Wiley mill, then a sub-sample was taken for chemical analysis. Total P was determined by ashing a subsample at 480°C, digesting in HNO_3 acid, and analyzing on a JY Ultima Inductively Coupled Plasma Spectrophotometer (Deal et al. 1996). N:P molar ratios were calculated for each species sampled for foliar analysis. A mean of these ratios was calculated for each elevation class and disturbance type.

Forest floor (O horizons + wood) was sampled on all plots during March 2010 for determination of total mass and P pools. Five samples per plot were collected with a 0.09 m^2 quadrat of forest floor separated into Oi, Oe, and Oa horizons and wood (< 10 cm diameter). Samples were processed and analyzed for P concentration similar to foliage samples. Forest floor and foliar samples were analyzed for total C and N by combustion on a Flash EA 1112.

Basal area increment (BAI) cores of the dominant overstory species were taken in October 2010, processed, and analyzed with methods previously stated in Chapter 1. BAI ratios and the pre- and post-infestation slopes of those ratios (Chapter 1) were also used for analysis of P dynamics.

Soil and environmental measurements

I collected 20-25 soil cores at depths of 0-10 cm and 10-30 cm from random locations within each plot in June 2010. Samples were processed and analyzed similar to methods described in Chapter 1. Bulk density (g soil cm^{-3}) (both coarse fragment and < 2 mm fractions) was determined on soils from each plot using 4.3 cm diameter PVC cores to sample 0-10 cm and 10-30

cm depths. These data were used to calculate soil nutrient pools in kg ha^{-1}. Soil chemical analyses included total N by combustion on a Flash EA 1112, weak acid extractable PO_4, and 0.01 M $CaCl_2$ soil pH. Total and inorganic P was determined by shaking one gram soil overnight with 0.5M H_2SO_4 and analyzing on the JY Ultima Inductively Coupled Plasma Spectrophotometer (soil was muffled at 550°C for seven hours prior to shaking for total P). Organic P was calculated by subtraction (Deal et al. 1996).

Previously homogenized soil samples from both depths were analyzed using a modified version of the sequential P fractionation method developed by Psenner et al. (1988) (SanClements et al. 2009). Briefly, we sequentially extracted one gram samples into the following P fractions: (1) 1M NH_4Cl, pH 7, at 25°C for 1 hour was considered the exchangeable fraction; (2) 0.11M $NaHCO_3$-$Na_2S_2O_4$ (BD) at 40°C for 30 minutes was considered P adsorbed to reducible metal hydroxides, thought to be primarily Fe-bound P; (3) 0.1M NaOH at 25°C for 16 hours extracted P predominately associated with Al and some Fe hydroxides as well as organic P; (4) 0.5M HCl at 25°C for 16 hours extracted acid-soluble primary mineral P, considered to be apatite-P; and (5) 1M NaOH at 85°C for 24 hours to dissolve refractory and residual P. All extractions were centrifuged at 3000g for 15 minutes and filtered through Whatman 42 filter paper (Whatman Inc., Clifton, NJ). Extractions were then repeated with an agitation time of one minute to rinse samples. All extracts were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) at the Coweeta Hydrologic Analytical Laboratory.

Ion-exchange resin lysimeters (IERs) (Susfalk and Johnson 2002) were installed in all plots in April 2010. Eight IERs were randomly located throughout each plot; four below the forest floor ("forest floor lysimeters") and four below the major rooting zone ("mineral soil lysimeters"). To place the four mineral soil lysimeters, we dug two 50-cm deep pits with two channels perpendicular

from the bottom. Two IERs were placed at the ends of these channels; far enough away from the loose soil to ensure the soil column above the lysimeters remained undisturbed. During quarterly sampling (July 2010, October 2010, February 2011), both forest floor and mineral soil lysimeters were swapped with freshly prepared IERs, stored in individual bags, and kept cool during transport to the laboratory. Initial placement of all IERs was randomized by maintaining a distance of ~1-2 m away from any tree trunk or shrub.

Nylon resin bags were filled with 20 g of the same resin used in IERs (Rexyn I-300 HOH Beads- Research Grade/Certified) approximately one week prior to field installation. The bags were refrigerated and/or kept in a cooler before installation and during transport. In the field, a bulb planter was used to remove the top five cm of soil while a resin bag was placed at the base of this core, then the intact soil core and forest floor was replaced and marked with flagging. Five bags were randomly placed in the mineral soil surface in every plot (n=90) and during quarterly sampling when resin bags were switched out, a new hole just adjacent to the previous location was dug for each new resin bag. After removal from the field, resin from IERs and resin bags was extracted using 2M KCl, and the extract was filtered through 0.7 µm Whatman filter paper and frozen until analysis. Extracts of lysimeters and resin bags were run for dissolved reactive phosphorus (DRP) (Ortho-phosphate method) using a Lachat QuikChem 8000 (Hach Company, Loveland, CO).

Statistical analysis

Samples with a single collection date (foliar, forest floor, composite soil samples) were analyzed using ANOVA with elevation (low, mid, high) and disturbance (DH, REF) as main and interactive effects. I used Tukey's test to identify significant differences between groups within

class variables, and the Slice statement to evaluate simple main effects of fixed variables within interactions. For IER data with repeated collection dates, I used plot means for each temporal period and determined changes over time with a repeated measures statement using an unstructured covariance structure (un) in a generalized linear mixed model with plot (elevation × disturbance) as the subject. Data were logarithmically transformed as necessary to meet assumptions of normal distribution and equal variance. All analyses were done in SAS v.9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Wet inorganic N (NO_3-N + NH_4-N) deposition and N availability as measured by resin bags increased with elevation ($F_{18,2}$=34.41, p<0.0001; $F_{54,2}$=10.25, p=0.0025, resp.) during the study period (Table 2). High elevation plots had 2.69 ± 0.416 (s.e.) mg inorganic N (NO_3-N+NH_4-N) per 20 g resin bag compared to the smaller amounts observed in mid (0.523 ± 0.079) and low (0.304 ± 0.066) elevation plots.

Vegetation measurements

At the plot-level, the concentration of P in live foliage varied significantly with elevation ($F_{2,18}$=19.82, p=0.0002; Fig. 6a). Averaged over disturbance categories, mean foliar P concentrations were higher in high elevation plots (0.75 ± 0.14 SE) than in mid- and low elevation plots (0.16 ± 0.10 and 0.02 ± 0.005, resp.) (Tukey's HSD: p<0.01). Disturbance also affected plot-level foliar P concentrations ($F_{1,18}$=5.37, p=0.0389), such that DH plots generally had higher foliar P than REF plots (Fig. 6a). However, the degree of difference between DH and REF plots varied with elevation. Analysis of simple effects of disturbance within each elevation class indicated foliar

P was significantly higher in DH than REF plots at high elevations ($F_{1,6}$=5.80, p=0.0330) but not at mid- or low elevations. Foliar N:P ratios also varied by elevation ($F_{2,54}$=14.44, p<0.0001), but nonlinearly, such that mid-elevation plots had significantly higher N:P ratios (mean ± SE: 40.5 ± 1.7) than low (33.9 ± 1.2) and high elevation plots (31.3 ± 0.9). Disturbance, however, had a strong, consistent effect across all elevations, such that foliar N:P ratios were significantly lower in DH plots compared with REF plots ($F_{1,54}$=5.08, p=0.0290) (Fig. 6b). There was no evidence that the influence of disturbance on foliar N:P ratios varied with elevation; interactions were non-significant.

Total forest floor (Oi + Oe + Oa + wood) P (kg ha^{-1}) also increased with elevation ($F_{2,18}$=21.21, p=0.0001) (Table 3). This relationship was apparent in both DH ($F_{2,9}$=8.45, p=0.0051) and REF stands ($F_{2,9}$=13.65, p=0.0008). Disturbance, however, had no effect on forest floor P and did not interact with elevation (data not shown). Concentration of P (μg P g^{-1}) in forest floor horizons had similar results; elevation had positive effects on Oi ($F_{2,18}$=15.46, p=0.0005), Oe ($F_{2,18}$=5.09, p=0.0164), Oa ($F_{2,18}$=24.78, p=0.0002), and wood ($F_{2,18}$=8.63, p=0.0048) (Table 3). Again, no differences were seen between disturbance types and the interaction of elevation × disturbance was not significant.

Soil and environmental measurements

Total P pools as measured by P fractionation (P_{SUM}) were not significantly different across the basin in surface or deep soils (Table 4, Fig. 7). All soils tested had effectively no P_{NH4Cl} and low amounts of the second extracted fraction, $P_{BD.}$ A weak acid extraction was done to more accurately assess plant-available P; although this fraction did decrease with elevation in the top 10 cm, the

trend was not significant, and there was no apparent decrease in the 10-30 cm depth. There was also no difference with disturbance and no interaction of the main effects (Table 4).

Overwhelmingly, the largest pool of soil P was in the $P_{NaOH\text{-}25}$ fraction, where P is bound to Al, Fe, or in organic form. This fraction increased with elevation in the top 10 cm ($F_{2,18}$=6.26, p=0.0137) but was similar across elevations in deeper soils (Fig. 7). There were no differences observed with disturbance and no interaction of the main effects in either depth in the $P_{NaOH\text{-}25}$ fraction. The P_{HCl} fraction, thought to be primarily mineral P (apatite), declined slightly with elevation in both depths, but the decrease was not significant, nor was the interaction of elevation × disturbance. In the top 10 cm, the P_{HCl} fraction did not vary with disturbance; however, in deeper soils, DH plots had moderately more P_{HCl} than REF plots ($F_{1,18}$=4.15, p=0.0644) (Fig. 7).

The next largest fraction after $P_{NaOH\text{-}25}$ was $P_{NaOH\text{-}85}$ (Table 4), the residual and recalcitrant pool of soil P which is generally thought to be unavailable to plants. In both depths, the size of $P_{NaOH\text{-}85}$ fraction decreased with elevation (0-10 cm: $F_{2,18}$=17.18, p=0.0003; 10-30 cm: $F_{2,18}$=17.65, p=0.0003) and Tukey-adjusted LS means statements showed that high elevations had much smaller pools than low (0-10 cm p=0.0036; 10-30 cm p=0.0129) and mid (0-10 cm p=0.0003; 10-30 cm p=0.0002) elevation stands. There was neither a disturbance effect nor an elevation × disturbance interaction at either depth.

The majority of P in these two fractions ($P_{NaOH\text{-}25}$ and $P_{NaOH\text{-}85}$) was organic, as the muffled soil P extractions had relatively small inorganic pools (Fig. 8). Organic P increased dramatically with elevation ($F_{2,18}$=56.01, p<0.0001), but did not vary between disturbance types . The interaction of elevation × disturbance ($F_{2,18}$=4.23, p=0.0406) was driven by soils of high elevation REF stands having significantly higher concentrations of organic P compared to DH stands at the same elevation ($F_{1,6}$=10.42, p=0.0073). At these high elevations, REF stands had 47% larger pools of

organic P in soils than DH stands ($F_{1,6}$=10.42, p=0.0072), while there were no differences with disturbance at mid or low elevations.

Flux of DRP from the forest floor increased with elevation ($F_{2,54}$=6.33, p=0.0140), but did not differ between DH and REF stands (Fig. 9). Over the entire sampling period, forest floor DRP flux increased with elevation in DH stands ($F_{2,27}$=5.46, p=0.0206), but was similar across REF stands in all elevations. However, the difference in forest floor DRP flux between stand types was apparent at high elevations, where DH stands had higher DRP flux compared to REF stands ($F_{1,18}$=5.47, p=0.0347) over the 9 month sampling period. Fluxes also varied with temporal period ($F_{2,54}$=46.94, p<0.0001); highest fluxes were observed in the late growing season (July-October) compared to the early growing season (April-July) (LS means, Tukey adj. p=0.0346) and the fall/winter period (October-February) (LS means, Tukey adj. p<0.0001). This is consistent with the timing of the growing seasons in this region.

Subsurface leaching of DRP was low across all sites (<0.21 kg ha^{-1} $9mo^{-1}$) and did not vary by elevation or disturbance type; temporal period explained most of the variation in DRP leaching ($F_{2,54}$=26.67, p<0.0001) (Fig. 9).

Discussion

As expected, N availability increased with elevation, and pools and fluxes of P responded to this increased availability with changes in soil, forest floor, and foliar P distributions, concentrations, and ratios. Working in the same sites, I also found that forest floor, soil, and fluxes of N increased with elevation (Chapter 1). These findings are consistent with those of Knoepp et al. (2008) who found that high elevation stands had increased litterfall N, faster rates of soil N

transformations, higher soil solution N, and greater stream exports of N compared to low elevation stands within the Coweeta basin.

At the same time, my data provide no evidence that higher N availability has resulted in P limitation in this region. Total soil P based on summed Psenner fractions did not vary with elevation in the 0-10 cm or 10-30 cm depths. Similarly, the weak acid extraction of P, an index of plant-available P, did not significantly decrease with elevation or vary by disturbance type. These findings are consistent with those of other studies examining effects of N availability on P dynamics. Weand et al. (2010) studied forest plots in the Catskill Mountains of New York that had been fertilized with N for 10 years and examined how P characteristics differed among tree species and/or responded to simulated N deposition. Measures of foliar, litter, and root P concentrations, as well as soil P availability and biotic P sufficiency all differed by species but were unaffected by N fertilization (Weand et al. 2010). Similarly, Finzi (2009) evaluated basal area growth of sugar maple/white ash stands with high N availability and oak/beech/hemlock stands with low N availability in New England and their response to fertilization with N and P. Several tree species responded to fertilization with N alone and only one species responded to P fertilization once N was added, suggesting that decades of atmospheric N deposition have not (yet) resulted in widespread P limitation or saturation of tree demand for N (Finzi 2009).

It is possible that levels of atmospheric N deposition received over the study period were not sufficient to induce P limitation. Levels of total wet N deposition were much smaller (3.40-4.44 kg ha^{-1} $9mo^{-1}$) than those reported by Mohren et al. (1986) (>50 kg N ha^{-1} yr^{-1} in the Netherlands) who found evidence that increased N availability leads to P limitation. Likewise, Tessier and Raynal (2003) studied N:P ratios in the Catskill Mountains, and found signs of P limitation in the understory vegetation due to a decade of high N deposition which averaged 6.40 kg ha^{-1}annually.

Differences in stand composition among studies may also account for variation in P response to N availability. Weand et al. (2010) found that hardwood species vary in the degree to which they rely on internally stored and recycled P vs. P obtained from the soil. In their study, beech, birch, and maple trees had sufficient or average levels of foliar and litter P, while oaks had the highest foliar, litter, and available soil P, as well as the lowest P resorption (Weand et al. 2010). These oak-dominated plots had the highest concentration of inorganic P and high proportions of P bound in organic fractions, which suggests that P turnover may be relatively rapid and support the status of oaks as a "P rich" species (Weand et al. 2010). The plots contained these species, but several others as well (Table 2), whose P cycling traits are not as well known.

My results suggest is that greater N availability increased biotic demand for P, mobilizing P from recalcitrant pools. I found smaller residual and recalcitrant pools of P in high N available stands, and no significant decline in the apatite fraction, suggesting a restructuring of soil P pools once thought to be unavailable to biota apparently unrelated to weathering. These findings are consistent with those of Richter et al. (2006) who found that biotic P demand drove the depletion of slowly cycling inorganic P pools, thereby maintaining the labile soil P fraction and overall P availability. At high elevations, I also found higher soil organic P pools, higher P flux, and more P stored in foliage and forest floor wood, suggesting that P mined from recalcitrant mineral soil P pools is actively cycling through the system. Previous studies show that increased N availability can stimulate extracellular phosphatase which cleaves ester-P bonds in soil organic matter and can increase local P availability (McGill and Cole 1981, Olander and Vitousek 2000, Treseder and Vitousek 2001, Wang et al. 2007, Finzi 2009, Vitousek et al. 2010, Weand et al. 2010), which suggests a mechanism by which enhanced N may drive restructuring of soil P. Another possible mechanism explaining my results could be the coupled release of P when Al and Fe fractions were

liberated by very low soil pH levels associated with long-term elevated N (acidification-induced mobilization), as proposed by Sherman et al. (2006). In my study, soils at high elevations did have significantly lower pH values than at mid and low elevations (Table 2), but there were no differences between DH and REF stands.

The effects of hemlock decline on P pools and fluxes at high elevations especially, suggest that disturbance and N availability interact to affect P availability. Previous studies indicate that disturbance is crucial for rejuvenating available P (Fitzhugh et al. 2001, Frizano et al. 2002, Wardle et al. 2004). My results are among the first to show that N availability can enhance this effect by increasing the pool size of bioavailable phosphorus. The lower N:P ratios in disturbed stands indicate that hardwoods that once co-occurred with healthy hemlocks are taking up P made available by hemlock decline. In contrast, I found no difference in forest floor P content between DH and REF stands. One possible explanation for this is that trees readily re-sorbed P prior to leaf senescence. Post leaf drop, P may also have been rapidly reincorporated into microbial or plant biomass or soluble P may have leached from litter prior to sampling. Indeed, DRP flux was highest in disturbed stands during the late growing and fall/winter periods, consistent with the timing of leaf drop. Had litter been sampled throughout the year, differences among litter P values may have been detected. Regardless, the relatively larger surface DRP flux in disturbed stands across elevations suggests P is cycling more rapidly in stands experiencing hemlock decline than in REF stands. Slightly higher amounts of DRP leached from the soil in April-July from disturbed stands, but the amounts relative to the inputs at the soil surface are miniscule.

Working at the same sites, I found that mixed hardwoods in disturbed stands at high elevations had significantly higher rates of growth post-HWA infestation compared to those at low and mid elevations (Chapter 1), whereas prior to infestation growth rates were comparable. This

suggests that nutrients liberated by hemlock decline are increasing productivity. Observed increases in foliar N and decreases in subsurface N leaching further suggest that vegetation disturbance has alleviated proximate P limitation and is stimulating increased N uptake (Chapter 1). Nutrient limitation, however, cannot be evaluated solely on the basis of ratios of foliar nutrients, and fertilization studies are needed to understand the impacts of disturbance on productivity under conditions of chronically elevated N inputs.

Acknowledgements

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Table 2 Site characteristics for HWA-infested (DH) and reference (REF) plots at low (L), middle (M), and high (H) elevations, including dominant vegetation sampled for foliar analysis, elevation (m), pH (0.1M $CaCl_2$), atmospheric wet inorganic nitrogen deposition (kg NH_4-N + NO_3-N ha^{-1}) measured April 2010-December 2010, total plot basal area (live), soil series and classification, and a description of parent material

Site	Dominant vegetation	Elevation	pH*	N dep.	Total live basal area	Soil classification	Parent material
		m	0.1M $CaCl_2$	kg ha^{-1} $9mo^{-1}$	m^2 0.04 ha^{-1}		
DH-L	*Acer rubrum, Betula lenta, Ilex opaca, Liriodendron tulipifera, Nyssa sylvatica, Oxydendrum arboreum, Quercus alba*	710-716	4.15 4.18	3.45 ± 0.06	56.08 ± 8.12	Saunook series: a fine-loamy, mixed, superactive, mesic Humic Hapludults	Colluvium derived from materials weathered from felsic to mafic, igneous and high-grade metamorphic rocks
						Cullasaja-Tuckasegee complex: a fine-loamy/loamy-skeletal, isotic, mesic Typic Humudepts	Colluvium derived from materials weathered from felsic to mafic high-grade metamorphic and igneous rocks
REF-L	*A. rubrum, B. lenta, Carpinus caroliniana, Fagus grandifolia, L. tulipifera, Q. alba, Quercus velutina*	698-715	4.33 4.43	3.40 ± 0.06	50.83 ± 10.78	Evard-Cowee complex: a fine-loamy, parasesquic, mesic Typic Hapludults	Residuum affected by soil creep in the upper part and weathered from felsic to mafic, igneous and high-grade metamorphic rocks
						Fannin series: a fine-loamy, paramicaceous, mesic Typic Hapludults	Residuum affected by soil creep in the upper part, and weathered from high-grade metamorphic rocks that are high in mica content

DH-M	*A. rubrum, B. lenta, Carya spp., L. tulipifera, N. sylvatica, O. arboreum*	917-934	4.17 4.24	3.96 ± 0.20	62.79 ± 2.56	Evard-Cowee complex Cullasaja-Tuckasegee complex	See above See above
REF-M	*A. rubrum, Carya spp., L. tulipifera, O. arboreum, Quercus coccinea, Q. velutina, Robinia pseudoacacia*	924-943	4.18 4.31	4.12 ± 0.22	47.68 ± 0.97	Evard-Cowee complex Cullasaja-Tuckasegee complex	See above See above
DH-H	*Acer pensylvanicum, A. rubrum, Betula alleghaniensis, Fagus grandifolia, Quercus rubrum*	1402-1433	3.95 4.15	4.44**	84.91 ± 7.94	Plott series: a fine-loamy, isotic, mesic Typic Humudepts	Residuum affected by soil creep in the upper part and weathered from felsic to mafic igneous and high-grade metamorphic rocks
REF-H	*A. pensylvanicum, A. rubrum, Acer saccharum, B. alleghaniensis, Ostrya virginiana, Prunus serotina*	1411-1423	3.98 4.16	4.44**	59.74 ± 7.54	Plott series	See above

* 0-10 cm, 10-30 cm depths, ** only one rain gauge was used at high elevations.

Table 3 Forest floor mass and concentrations for HWA-infested (DH) and reference (REF) plots at low (L), mid- (M), and high (H) elevations. Values are means (± 1 SE).

	Total mass ($g\ m^{-2}$)	Carbon ** ($kg\ ha^{-1}$)	C:N molar ratio***	Phosphorus concentration (µg / g)			
				Oi**	Oe*	Oa**	Wood*
DH-L	858 (246)	15,073 (6,050)	62 (3.2)	658 (88.7)	606 (39.4)	842 (68.9)	199 (50.6)
REF-L	409 (96.9)	5,585 (404.3)	55 (2.2)	488 (114)	825 (63.2)	n/a	280 (7.10)
DH-M	450 (90.1)	7,567 (955.3)	48 (4.8)	595 (55.3)	713 (43.7)	737 (37.5)	315 (45.1)
REF-M	536 (179)	9,477 (1,908)	45 (3.5)	423 (33.3)	664 (47.0)	752 (70.6)	287 (14.3)
DH-H	1,411 (506)	20,925 (1,378)	29 (2.1)	955 (138)	1,012 (72.92)	1,254 (143.7)	379 (54.9)
REF-H	1,407 (553)	18,392 (2,208)	26 (0.7)	958 (39.1)	949 (193)	1,563 (111.6)	445 (52.7

Significant main effect of elevation (*$\alpha \leq 0.05$, **$\alpha \leq 0.01$, ***$\alpha \leq 0.0001$). No effect of disturbance or any interactions were observed.

Table 4 Soil P fractions and a weak acid extraction (kg P / ha). P_{NH4Cl} is labile-P, P_{BD} is reducible iron-P, $P_{NaOH-25}$ is P bound to organic, Al, and some Fe-P, P_{HCl} is exposed apatite P, $P_{NaOH-85}$ is refractory and residual-P, and P_{SUM} is the sum of all extractable P fractions.

Soil P (kg ha-1)	Low elevations		Mid elevations		High elevations	
0-10 cm	**DH**	**REF**	**DH**	**REF**	**DH**	**REF**
Weak acid extraction	6.18 (1.64)	6.03 (1.57)	5.84 (1.17)	4.15 (0.74)	4.11 (0.90)	3.22 (0.34)
P_{NH4Cl}	BDL	BDL	BDL	BDL	BDL	BDL
P_{BD}*	6.64 (0.40)	5.63 (0.46)	4.97 (0.31)	3.63 (0.35)	5.34 (0.90)	5.47 (0.28)
$P_{NaOH-25}$*	337 (81.3)	259 (32.1)	264 (49.4)	202 (12.3)	398 (81.3)	484 (71.0)
P_{HCl}	5.52 (0.64)	5.00 (0.94)	4.81 (0.57)	4.36 (0.58)	4.60 (1.33)	3.93 (0.34)
$P_{NaOH-85}$**	123 (9.45)	132 (0.92)	195 (52.7)	178 (47.7)	54.4 (9.90)	61.4 (10.7)
P_{SUM}	472 (90.2)	402 (32.9)	469 (35.0)	388 (49.9)	462 (89.3)	555 (81.4)
10-30 cm	**DH**	**REF**	**DH**	**REF**	**DH**	**REF**
Weak acid extraction	7.40 (0.40)	8.14 (0.77)	7.81 (2.21)	6.90 (1.07)	8.79 (1.36)	7.23 (0.47)
P_{NH4Cl}	BDL	BDL	BDL	BDL	BDL	BDL
P_{BD}	6.97 (0.84)	4.19 (0.62)	9.10 (1.69)	7.21 (2.14)	8.36 (2.04)	11.3 (3.65)
$P_{NaOH-25}$*	811 (115)	646 (21.0)	706 (53.9)	566 (8.65)	923 (234)	1143 (191)
P_{HCl}	15.2 (0.70)	11.2 (0.67)	13.2 (1.61)	14.0 (1.17)	14.6 (4.60)	7.36 (0.38)
$P_{NaOH-85}$**	332 (3.66)	297 (32.9)	722 (342)	607 (154)	148 (29.2)	132 (23.5)
P_{SUM}	1165 (119)	958 (52.3)	1451 (343)	1195 (153)	1094 (258)	1293 (218)

Notation for significant main effects of elevation (* $\alpha \leq 0.05$, ** $\alpha \leq 0.01$). Standard errors are in parenthesis (±); values below the detection level of the analytical instrument are noted as BDL.

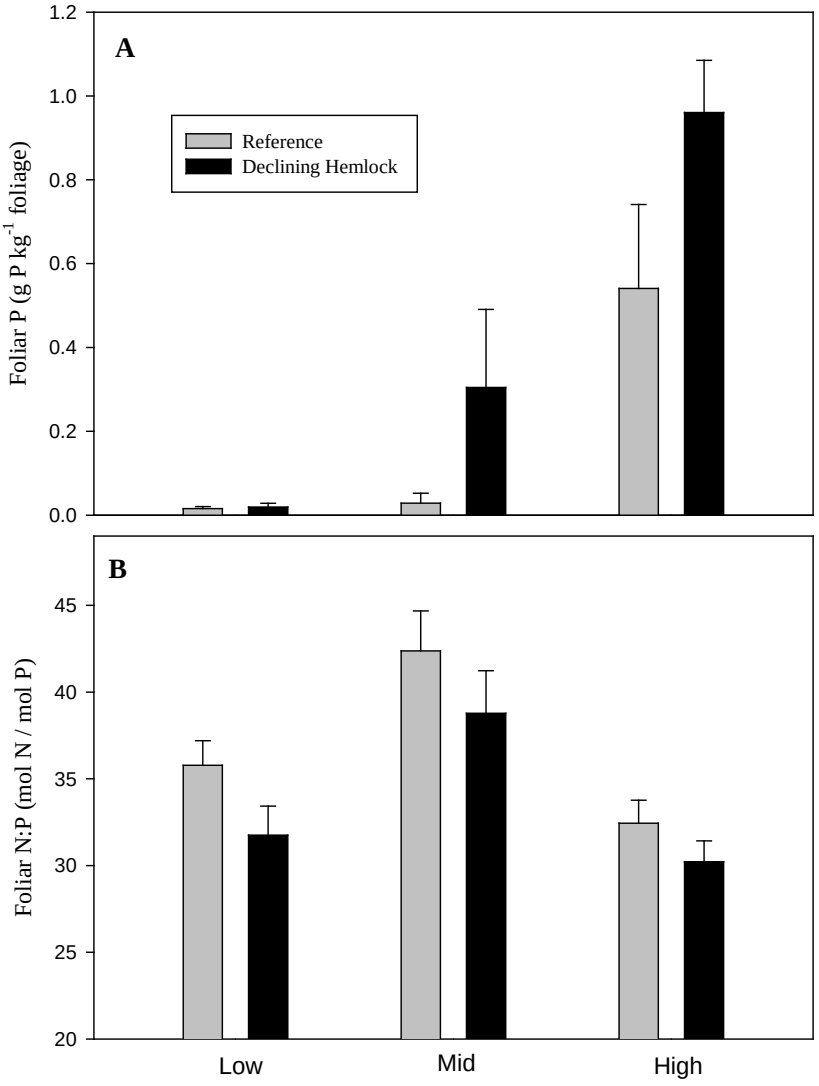

Figure 6 Foliar P concentration weighted by the relative foliage mass of each species within a plot (A) and foliar N:P ratios (mol N / mol P) (B).

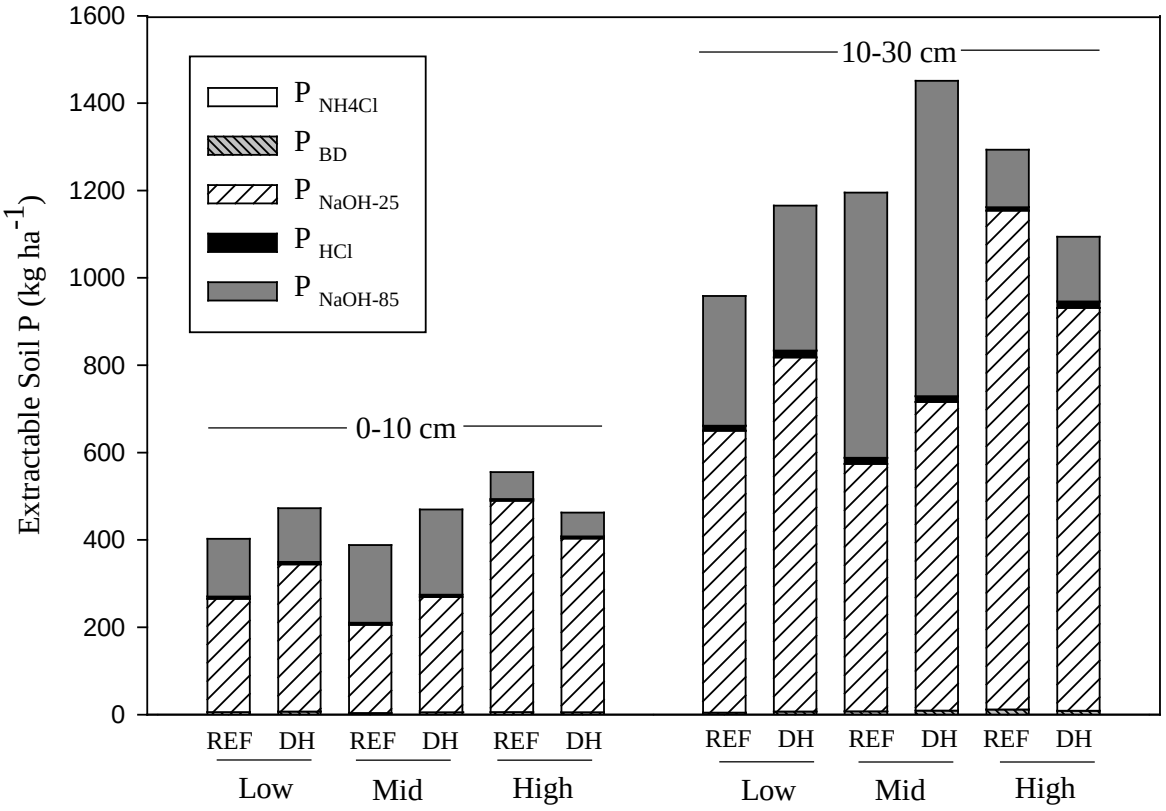

Figure 7 Soil phosphorus (P) in stands with declining hemlock (DH) and reference (REF) stands containing a similar composition of hardwoods in western North Carolina. A modified version of Psenner P fractionation (SanClements et al. 2009) was performed on soil from two depths at each of these vegetation types at three elevations within and around the Coweeta Hydrologic Laboratory.

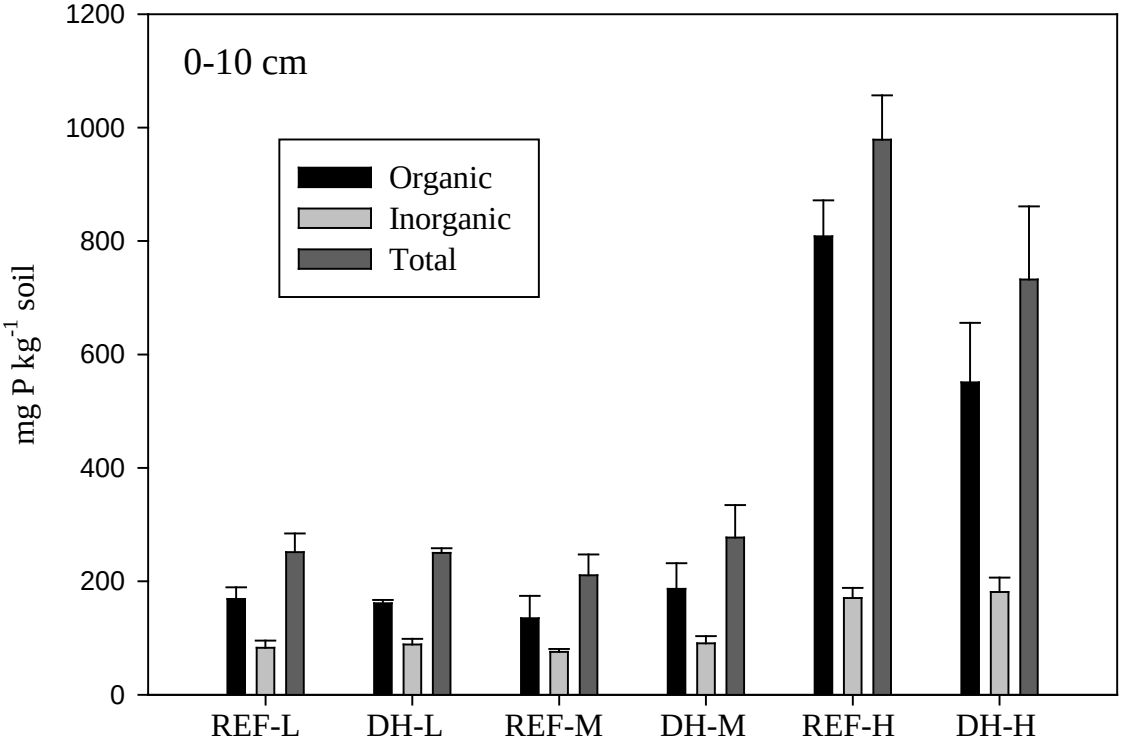

Figure 8 Total and inorganic P concentration (mg P / kg soil) (organic P by subtraction) from a sulfuric acid extraction on the top 10 cm of soil in declining hemlock (DH) and reference (REF) stands at three elevations in western North Carolina.

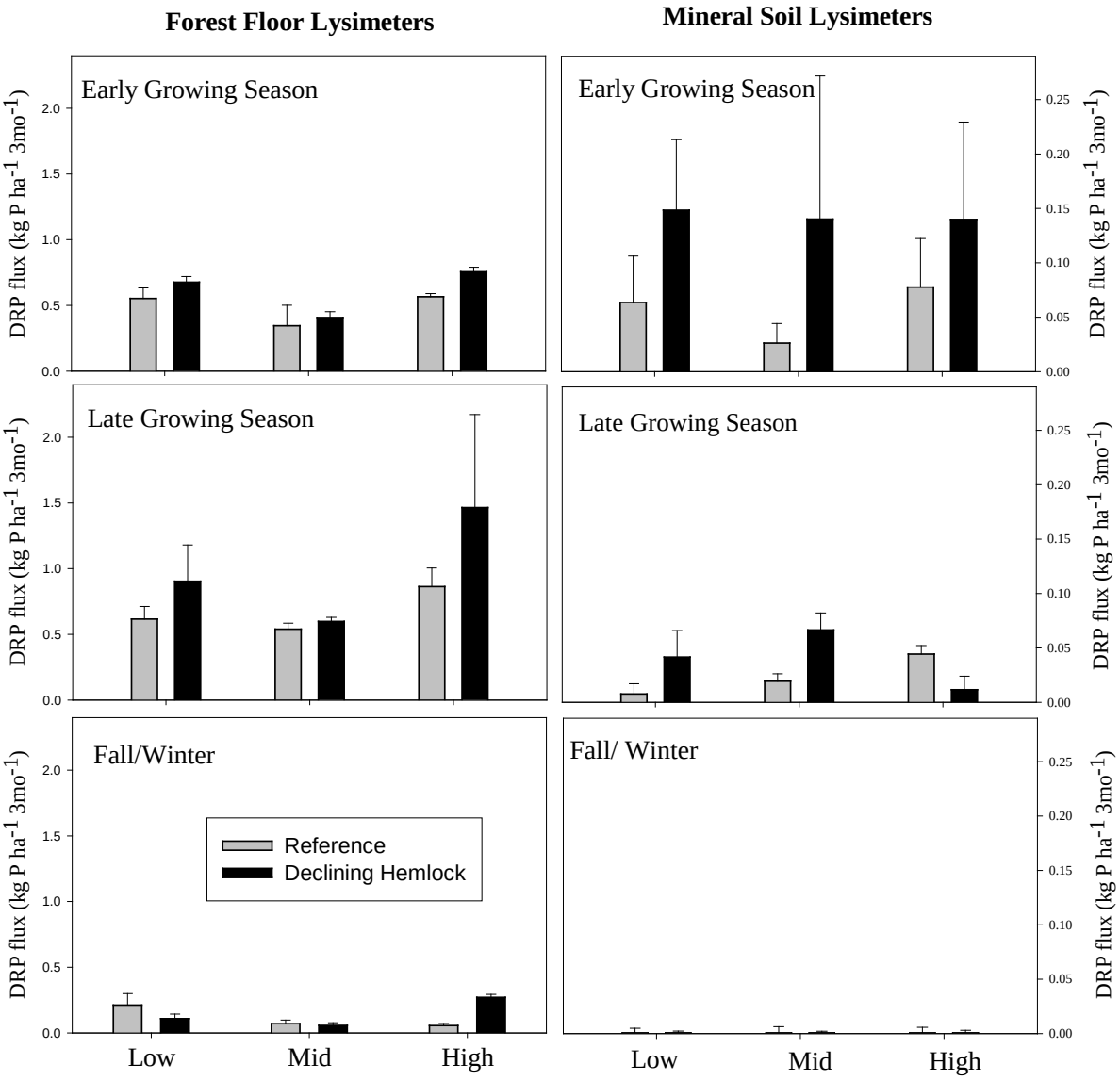

Figure 9 Dissolved reactive phosphorus (DRP) flux (kg DRP ha^{-1} 3 mo^{-1}) for forest floor and mineral soil lysimeters in stands with declining hemlock and reference hardwood stands for each temporal period (early growing season, April-July 2010; late growing season, July-October 2010; fall/winter, October 2010-February 2011) across all elevation classes.

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Conclusion

Purpose

This study enhances understanding of how biotic and abiotic variables interact; it also helps enhance our understanding of how disturbances and biogeochemical cycles interact, as well as the ecosystem factors that contribute to the magnitude of observed responses. Eastern hemlock and HWA were selected as the focal pest-host system because hemlock strongly regulates nutrient retention in forests and HWA is host specific. In the southeastern United States, the rate of HWA spread has been faster than the observed rate in the northeast, and here, hemlock decline may be observed on much larger scales than has previously been seen in the northern regions. On the other hand, hemlock is a sub-dominant species in the southeastern US and may not induce the drastic ecosystem changes observed in the New England area where much of the previous research has been done. In many forest stands, hemlock is abundant in riparian areas and may be the only tree species that cycles water and nutrients year-round. Therefore, even though hemlock is not the dominant species throughout the forests of the southeast, its loss will likely change stream habitats and may initiate irreversible changes in ecosystem function.

At high N-available sites, biotic sinks were strong and incorporated excess nitrogen (N) and phosphorus (P) into their biomass. Soil C:N ratios also declined with elevation, indicating that changes in N availability driven by N deposition play a strong role in the biogeochemical cycling in this region. Furthermore, at high N-available sites, stands experiencing hemlock decline increased their growth rates, foliar N and P, and fluxes of P from the forest floor to the soil profile. In reference stands, the flux of N increased in response to N deposition as expected, however, in stands with declining hemlock, leaching amounts were lower than expected and the

observed patterns varied seasonally. Perhaps lower elevation sites with low-N availability are subject to retention, or the longer growing season affects these interactions. It is also highly likely that they are co-limited by P or another ecosystem factor (i.e. water). There are likely multiple factors that influence these interactions and the magnitude of response.

Limitations

Ideally, ecosystem pools and fluxes would have been sampled more thoroughly: all trees within a stand would have been measured for foliar nutrients, and flux would have been measured year-round, preferably for several years. Sampling forest floor several times throughout the year would have given me a more complete view of nutrients moving from biotic pools to the soil profile. Sampling the flux of organic N and the microbial communities living in these stands would likely provide extremely interesting results that may help explain some of the observed patterns and extreme variability I observed throughout the study. Pairing this study along a similar elevation gradient in the northeast may have confirmed some of the conclusions I came to regarding mixed hemlock/hardwood stands versus pure hemlock stands.

Future work

My findings from P fractionation data are supported by strong, yet limited research; knowledge on P cycling in relation to increasing N availability in forest ecosystems is extremely limited. Because P is an ultimate limiting nutrient and may hinder forest productivity equally or more so than N, studies investigating the effects of disturbances and increasing N availability on P cycling is crucial.

The controls on impacts from pest-induced tree decline will likely depend on the strength of biotic sinks and how the nutrient cycling processes of these mixed deciduous forests interact with abiotic variables such as N availability. Magnitudes of impacts may depend on the unique species or mix of species found within each stand and their potential to be strong biotic sinks for changing nutrient landscapes. If exotic, invasive species become a more widespread, persistent problem in these forests, the need to understand the controls on the magnitude of ecosystem effects is invaluable. Nutrient limitation, however, cannot be evaluated solely on the basis of ratios of foliar nutrients, and fertilization studies are needed to understand the impacts of disturbance on productivity under conditions of chronically elevated N inputs.

Appendix A

Dominant hardwood species sampled in each plot in declining hemlock (DH) and reference (REF) stands at low (L), mid (M), and high (H) elevations within and around Coweeta Hydroloic Laboratory in western North Carolina.

Site	Plot	Species	Foliar %N	Foliar %P	Foliar N:P (mol N / mol P)	Total species basal area within a plot (m^2)	Relative basal area (spp BA/ total plot BA) (%)
DH-L	1	*Acer rubrum*	2.02	0.138	32	0.55	4.54
		Oxydendrum arboreum	1.81	0.135	30	1.09	9.09
	2	*Betula lenta*	2.50	0.146	38	6.19	46.8
		Liriodendron tulipifer	2.13	0.170	28	3.17	23.9
		Nyssa sylvatica	1.64	0.153	24	0.14	1.05
	3	*Ilex opaca*	1.17	0.069	38	0.40	6.07
		Liriodendron tulipifer	1.99	0.135	33	0.81	12.4
		Quercus alba	2.12	0.150	31	0.79	12.7
REF-L	1	*Acer rubrum*	1.51	0.115	29	1.43	17.4
		Fagus grandfolia	1.94	0.113	38	0.75	9.14
		Quercus alba	2.22	0.135	36	4.67	56.8
	2	*Acer rubrum*	1.87	0.120	35	1.35	26.5
		Betula lenta	2.56	0.164	35	0.60	11.8
		Carpinus caroliniana	1.94	0.118	36	1.97	38.3
	3	*Liriodendron tulipifer*	2.76	0.137	45	5.46	52.0
		Quercus alba	2.16	0.143	33	0.79	7.49
		Quercus velutina	2.02	0.127	35	2.43	23.1
DH-M	1	*Carya spp.*	2.19	0.101	48	1.44	18.9
		Nyssa sylvatica	1.71	0.106	36	1.20	15.8
		Oxydendrum arboreum	2.01	0.094	47	0.64	8.46
	2	*Betula lenta*	2.28	0.133	38	0.31	4.22
		Carya spp.	1.91	0.101	42	0.66	9.06
		Liriodendron tulipifer	2.25	0.126	40	2.41	33.3
	3	*Acer rubrum*	1.54	0.134	25	1.00	5.80

		Carya spp.	2.18	0.116	42	1.05	6.14
		Liriodendron tulipifer	2.40	0.172	31	6.10	35.5
REF-M	1	*Acer rubrum*	1.90	0.140	30	0.56	11.3
		Liriodendron tulipifer	2.15	0.111	43	1.14	22.8
		Oxydendrum arboreum	1.90	0.100	42	1.01	20.2
	2	*Acer rubrum*	1.74	0.086	45	1.04	16.7
		Liriodendron tulipifer	2.15	0.106	45	2.75	44.0
		Quercus velutina	2.64	0.086	68	0.41	6.62
	3	*Acer rubrum*	1.54	0.096	36	1.99	33.8
		Carya spp.	2.15	0.094	50	0.36	6.03
		Quercus coccinea	1.97	0.091	48	0.90	15.3
DH-H	1	*Acer rubrum*	1.54	0.120	28	2.22	10.7
		Fagus grandfolia	2.09	0.142	33	11.90	57.2
		Quercus rubra	2.08	0.137	34	4.62	22.2
	2	*Acer pensylvanicum*	2.04	0.177	26	0.03	0.12
		Betula alleghaniensis	2.44	0.172	32	18.50	82.6
		Quercus rubra	2.53	0.193	29	0.72	3.24
	3	*Acer pensylvanicum*	1.90	0.158	27	1.05	3.98
		Betula alleghaniensis	2.41	0.198	27	23.02	86.8
		Quercus rubra	2.83	0.174	36	0.57	2.16
REF-H	1	*Acer pensylvanicum*	2.04	0.144	31	1.14	8.82
		Betula alleghaniensis	2.44	0.146	37	9.83	76.3
		Prunus serotina	2.89	0.181	35	0.24	1.84
	2	*Acer saccharum*	2.15	0.151	31	0.77	4.44
		Betula alleghaniensis	2.07	0.176	26	14.23	82.3
		Ostrya virginiana	2.43	0.143	38	1.47	8.52
	3	*Acer rubrum*	1.87	0.146	28	1.30	13.0
		Betula alleghaniensis	2.20	0.154	32	0.86	8.64
		Prunus serotina	2.18	0.140	34	3.38	33.8